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Research Article
Evaluation of the Potential of *Trichoderma harzianum* as a Plant Growth Promoter and Biocontrol Agent Against *Fusarium* Damping-off in Onion in Burkina Faso

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Abstract

Background: This study is about an assessment of plant growth promoter and biocontrol properties of *Trichoderma harzianum* in onion. Two isolates of *T. harzianum* one originating from Eco-Tâ (South Africa) and the other from a onion field soil (Burkina Faso) were evaluated for their use as onion growth promoters and their efficiency in protecting onion seedlings against damping-off caused by *Fusarium oxysporum* and *F. solani*. **Materials and Methods:** *Trichoderma harzianum* was used for coating onion seeds with a conidial suspension concentrated to 10^7 spores mL^{-1} and for directly spraying onion seedbeds with the same conidial suspension. Sowing was done with coated seeds in trays containing a doubly sterilized substrate inoculated or not with *Fusarium* strains. Non-coated seeds were sowed in the same substrate sprayed with *T. harzianum* conidial suspension and inoculated or not with *Fusarium* strains. All trays were kept in greenhouse for 1 month. **Results:** Coating the seeds and spraying the seedbeds with the conidial suspension of the two *T. harzianum* isolates led to a significant increase in the number of living seedlings, seedling length, root length, number of leaves and fresh weight of seedlings 30 days after sowing in sterilized soils. Sowing the *T. harzianum* coated seeds in soils inoculated with *Fusarium* led to a significant reduction in seedling damping-off due to the pathogens and improved the growth parameters of seedlings compared with non-coated seeds. Spraying the conidial suspension of *T. harzianum* isolates on seedbeds inoculated with both *Fusarium* strains produced the same results, but at a lower intensity than was the case with seed coating. **Conclusion:** These results reveal that the use of an indigenous isolate of *T. harzianum* originated from Burkina Faso could be a novel biocontrol strategy against the damping-off of onion seeds in an environmentally sustainable way. This study discusses further development of a simple and accessible method for the mass production and conservation of *T. harzianum* and the search for an effective and inexpensive sticker for seed coating. An isolate of *T. harzianum* native to Burkina Faso was shown to improve the germination of onion seeds and to protect seedlings against *Fusarium* damping-off. This isolate can be exploited in the development of integrated onion crop protection in West Africa.

Key words: *Trichoderma harzianum*, seed coating, conidial suspension, onion, damping-off

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Among the vegetable crops produced in Burkina Faso, onion bulbs are the most important in terms of international trade. In 2008, for example, the income produced by this trade was evaluated at 24.8 billion CFA[1]. The level of income obtained by onion farmers is estimated[2,3] to be about US$ 5,000. Given this high level of farmer income, onion production and marketing have become a powerful tool in the fight against poverty and rural unemployment[4]. The onion sector is now a priority in the country's agricultural policy and is supported by the World Bank through two major projects, Programme d'Appui aux Filières Agro-Sylvo-Pastorales (PAFASP) and Projet de Productivité Agricole en Afrique de l'Ouest (PPAAO).

As in other countries, however, onion production is challenged by several diseases occurring in nurseries, in fields and during storage. Symptoms such as seedling damping-off, foliar lesions and bulb rot have been observed. These diseases result in the need to buy seeds (that are very expensive) and in yield losses that can sometimes[5] reach 80%. Fungi are among the plant pathogens that cause several diseases and significant yield losses worldwide. Most of these plant diseases, including seedling damping-off, blight, necrosis, wilt and rot are caused by oomycètes, ascomycetes and deuteromycetes fungi[6,7]. In Burkina Faso, *Fusarium oxysporum* and *F. solani* are associated mainly with the onion seed damping-off complex[8].

Disease control is carried out mainly through the use of chemicals. Given the limited availability of cheap and effective fungicides and the risks associated with their use (risks to farmer's health, problems caused by chemical residues on crops and in the environment, pathogen resistance to chemical), the search for alternative ways of combating plant pathogens is urgent and of worldwide concern[9-13]. Biological control represents an alternative means of controlling plant disease that reduce dependence on chemicals[14]. A wide range of biological control agents including bacteria, yeast and fungi have been investigated for use in plant disease control[15-18]. *Trichoderma* species are common soil saprophytic ascomycetes fungi from the family of Hypocreaceae; they are found in all climates throughout the world[19,20]. Some members of this genus have been investigated for their antagonism against various plant pathogens and their growth promotion potential and have been considered as sources for many biotechnological applications[14,17,21,22]. The capacity of *Trichoderma* species to control plant pathogens has been known since 1920; these fungi were first used as commercial biological control agents[23] in 1990. Several formulations based on *Trichoderma* have been patented and are now commercially available pesticides[24]. *Trichoderma harzianum*, in particular, has been reported to control damping-off caused by *Fusarium* species[25]. Some formulations based on actively growing hyphae on bran, wheat-bran-peat or maize perlite media have been successfully applied to the soil[26]. Such formulations, however, require large amounts of material that cannot be stored practically and applied because of their bulk. The use of antagonistic fungi in seed treatments is thought to be an economical alternative, because only relatively small amounts of inoculum are needed[26]. The successful application of *T. harzianum* through industrial seed coating against damping-off in sugar beet in the field has been reported by De Algaba *et al.*[27]. Afterwards, this species became the active component in a number of commercially available products, such as PlantShield® and RootShield® Granules (Bioworks Inc, New York, USA), developed for root disease suppression and plant growth promotion[28,29]. The GHE Bioponic-Mix is a formulation based on *T. harzianum* and marketed as an excellent plant growth promoter by the Spanish company Growshop Alchimia[30].

Velivelli *et al.*[31] observed that, for consistency of performance in the field, biocontrol agents need to be tested under different climatic conditions and on different crops against a range of pathogens in order to evaluate their potential for broad-spectrum activity. In this study, the antagonistic effects were tested of five isolates of *T. harzianum* obtained from soil samples from onion production areas in Burkina Faso (4) and one commercial product originating from South Africa (1) against *Fusarium oxysporum* and *F. solani in vitro*. One isolate from Burkina Faso and the one from South Africa inhibited the mycelial growth of the pathogens by more than 80% and exhibited mycoparasitism on the pathogens[32]. This study aimed at assessing the effect of coating onion seeds or spraying onion seedbed with an isolate of *T. harzianum* native to Burkina Faso on plant growth promotion and on the control of *Fusarium* damping-off under greenhouse conditions, in comparison with an isolate from a commercial product.

MATERIALS AND METHODS

***Fusarium* strains and *T. harzianum* isolates tested:** Two isolates of *T. harzianum*, one from Burkina Faso and the other from South Africa were tested in this study (Table 1). The isolate ThBFA from the village Tabtenga in Burkina Faso was isolated from soil sample collected from the rhizosphere of onion. Its isolation was done by soaking 10 g of soil in 100 mL of sterilized water. The homogenate was filtered through cloth

Table 1: Characteristics and origins of the tested *Fusarium* isolates and *Trichoderma harzianum* strains

Fungal species	Accession No.	Source	Variety	Provenance	Year of recovery
Fusarium oxysporum	8126 (Fo_{20-013})	Bulbs	Violet de Galmi	Mogtédo	2013
	8125 (Fo_{27-013})	Roots	Violet de Galmi	Solenzo	2013
Fusarium solani	8124 (Fs_{45-013})	Roots	Violet de Galmi	Sourou/Débé	2013
	8127 (Fs_{44-014})	Roots	Violet de Galmi	Di	2014
Trichoderma harzianum	8129 ($ThBFA_{2-014}$)	Soil	-	Tabtenga	2014
	8128 ($ThAFS_{4-014}$)	Eco-T powder	-	South Africa	2014

and the resulting aqueous solution was diluted 100 and 1,000 times. Some droplets of each aliquot were placed in Petri dishes containing Potato Dextrose Agar (PDA) medium. The petri dishes were then sealed with parafilm and incubated at 25°C under an alternative cycle of near ultra-violet light (NUV) and darkness (12/12 h) for 5 days. The isolate ThAFS was obtained from a commercial product named Eco-T, developed by Plant Health Products in South Africa, by placing small portions of the commercial powder in petri dishes filled with PDA medium, sealed and incubated. Fungal colonies obtained were purified in other petri dishes.

Strains of *Fusarium* were obtained from naturally infected onion organs (Table 1). Fragments of infected tissues were initially disinfected twice with ethanol for 30 sec and then with hypochlorite of sodium (1%) for 1 min. After disinfection, they were rinsed three times with distilled water and dried in aseptic conditions. After drying, they were transferred to petri dishes filled with potato dextrose agar medium (PDA) and incubated at 25°C under a light-dark cycle (12/12 h) for 7 days. The colonies obtained were purified in other petri dishes.

All the tested fungi were identified initially by their morphological characteristics (colony colours, phialids and conidia forms). This morphological identification was then confirmed by molecular identification after PCR amplification and sequencing of some relevant/discriminant genes, internal transcribed spacers for *Trichoderma*[33] and elongation factor 1-α for *Fusarium*[34], in the Phytopathology Laboratory of Université Catholique de Louvain, Louvain-la-Neuve, Belgium.

Onion seed sample and seed disinfection: A seed sample (500 g) of the variety (Violet de Galmi) commercialized by NANKOSEM LLC, one of the main onion seed suppliers in Burkina Faso, was used in the study. 'Violet de Galm'i is the most widely grown variety in the country because of its flavor and long shelf life. The disinfection was done by soaking the seeds in sodium hypochlorite solution (1%) for 5 min. The soaked seeds were then rinsed three times with sterile water during 20 min each time.

Seedbed substrate and inoculation with fungal pathogens: The seedbed substrate was composed of a mixture of sand and mould (1/5, 4/5) sterilized twice with a 24 h interval for 20 min at 120°C. This substrate was placed in nursery trays, using 1.25 L $tray^{-1}$. Per nursery tray, two agar plates with 7 day old cultures of the tested pathogenic fungi were mixed with the substrate.

Seed and seedbed treatments with *T. harzianum* isolates and sowing: *Trichoderma harzianum* isolates were applied on seeds by coating and on the seedbed by spraying. For the onion seed coating, a sticker solution based on methyl cellulose (MC) 1% was used. The choice of this sticker solution was based on the results reported by Bardin and Huang[15]. The MC solution was prepared by dissolving 1 g of MC in 100 mL of boiling sterilized water. From a 7 day old culture of *T. harzianum* isolates grown on Potato Dextrose Agar (PDA), the conidial suspension was prepared with the MC solution and the concentration was adjusted to 10^7 conidia mL^{-1}. Disinfected onion seeds were immersed in the conidial suspension at a rate of 3 mL/100 seeds for 15 min and then dried under a laminar flow cabinet. The number of conidia per seed was determined by vortexing 10 coated seeds in 10 mL of sterile water for 1 min. The concentration of the resulting solution was evaluated three times using a THOMA cell. The number of conidia per seed was then determined based on the volume of the solution and the number of seeds soaked. The number of conidia per coated seed using this method was 3×10^5 conidia. The total number of conidia on 50 coated seeds was then estimated to be 1.5×10^7. The coated seeds were then sown in nursery trays filled with untreated substrate. Treatment of seedbed with *T. harzianum* was performed by spraying the substrate with 3 mL of a conidial suspension concentrated to 10^7 conidia mL^{-1} $tray^{-1}$ after the sowing of non-coated seeds. For both treatments, the seeds were individually sown in the nursery trays at a rate of 50 seeds per tray and arranged in 7 rows.

Experimental layout: A total of 26 treatments were performed (Table 2) in two trials. Each treatment was conducted in three trays, each tray being a repetition. In the first trial, *Fusarium oxysporum* from Solenzo (Fo_{27-013}) and *F. solani* from Débé (Fs_{45-013}) were used as pathogens. In

Table 2: Experimental design of the study

Substrate inoculation	No. of pathogen isolates	*Trichoderma harzianum* treatments	Attributed codes
Substrate inoculated with *Fusarium oxysporum*	Fo_{27-013}	Untreated seeds and substrate	Fo_{27-013} alone
		Seeds coated with *T. harzianum* from South Africa	Fo_{27-013} $ThAFS_{enr}$
		Seeds coated with *T. harzianum* from Burkina Faso	Fo_{27-013} $ThBFA_{enr}$
		Substrate sprayed with *T. harzianum* from South Africa	Fo_{27-013} $ThAFS_{asp}$
		Substrate sprayed with *T. harzianum* from Burkina Faso	Fo_{27-013} $ThBFA_{asp}$
	Fo_{20-013}	Untreated seeds and substrate	Fo_{20-013} alone
		Seeds coated with *T. harzianum* from South Africa	Fo_{20-013} $ThAFS_{enr}$
		Seeds coated with *T. harzianum* from Burkina Faso	Fo_{20-013} $ThBFA_{enr}$
		Substrate sprayed with *T. harzianum* from South Africa	Fo_{20-013} $ThAFS_{asp}$
		Substrate sprayed with *T. harzianum* from Burkina Faso	Fo_{20-013} $ThBFA_{asp}$
Substrate inoculated with *Fusarium solani*	Fs_{45-013}	Untreated seeds and substrate	Fs_{45-013} alone
		Seeds coated with *T. harzianum* from South Africa	Fs_{45-013} $ThAFS_{enr}$
		Seeds coated with *T. harzianum* from Burkina Faso	Fs_{45-013} $ThBFA_{enr}$
		Substrate sprayed with *T. harzianum* from South Africa	Fs_{45-013} $ThAFS_{asp}$
		Substrate sprayed with *T. harzianum* from Burkina Faso	Fs_{45-013} $ThBFA_{asp}$
	Fs_{44-014}	Untreated seeds and substrate	Fs_{44-014} alone
		Seeds coated with *T. harzianum* from South Africa	Fs_{44-014} $ThAFS_{enr}$
		Seeds coated with *T. harzianum* from Burkina Faso	Fs_{44-014} $ThBFA_{enr}$
		Substrate sprayed with *T. harzianum* of South Africa	Fs_{44-014} $ThAFS_{asp}$
		Substrate sprayed with *T. harzianum* from Burkina Faso	Fs_{44-014} $ThBFA_{asp}$
Uninoculated substrate	-	Untreated seeds and substrate	Blanco
		Seeds coated in methyl cellulose (MC) solution	MC (1%)
		Seeds coated with *T. harzianum* from South Africa	$ThAFS_{enr}$
		Seeds coated with *T. harzianum* from Burkina Faso	$ThBFA_{enr}$
		Substrate sprayed with *T. harzianum* from South Africa	$ThAFS_{asp}$
		Substrate sprayed with *T. harzianum* from Burkina Faso	$ThBFA_{asp}$

-: Not applicable

the second trial, *F. oxysporum* from Mogtédo (Fo_{20-013}) and *F. solani* from Di (Fs_{44-014}) were used. The other treatments were the same in the two trials.

Test monitoring, data collection and statistical analyses: The sown trays were placed in completely randomized blocks in a greenhouse with at 20-25°C, relative humidity 50% and 16 h photoperiod. The trays were watered daily for 30 days. From the date of the first appearance of the coleoptiles, the number of emerged seedlings was counted every 2 days for 2 weeks. The final evaluation was conducted 30 days after sowing (30 DAS) and involved:

- Counting the total number of surviving and normal seedlings per nursery tray
- Evaluating the total length, the root length and the number of leaves on 10 seedlings randomly chosen in each nursery tray
- Evaluating the total weight of seedlings from each nursery tray

Data obtained from substrate that had not been inoculated with pathogens were used to assess the plant growth promoting properties of *T. harzianum*. Data obtained from substrate inoculated with pathogens were used to show the degree to which the onion seedlings were protected against attacks by *Fusarium* species.

Collecting the data every 2 days made it possible to trace the curves of seedling emergence over time for all the treatments. Those from the final evaluation were used to calculate the different seedling growth parameters.

The number of seedlings emerged 30 DAS allowed the general damping-off rate (GDR) to be calculated as follows:

$$GDR = \frac{50\text{-}NLS}{50 \times 100}$$

Where:
50 = The No. of sown seeds
NLS = The No. of living seedlings

From the GDR, the damping-off rate due to the action of pathogens (DRP) of the treatments was calculated by subtracting from the GDR of each treatment, the GDR of uninoculated substrate being considered as due to another factor:

$$DRP\ (\%) = GDR_{treat}\text{-}GDR_{Blanco}$$

Where:

DRP = Damping-off rate due to pathogens
GDR_{treat} = General damping-off rate of a treatment
GDR_{Blanco} = General damping-off of the uninoculated substrate

All the means were compared by one-way analysis of variance (ANOVA) using the Duncan range test at 5%, performed with IBM SPSS Stat.23 software.

RESULTS

Growth promoter effect of *T. harzianum* isolates

Speed of seedling emergence: The number of emerged seedlings according to the *T. harzianum* treatment method and the sowing date are shown in Fig. 1 and 2. For all treatments, the seedling emergence speed curve consisted of four phases:

- A lag phase characterized by low daily emergence; this phase lasted from 0-5 DAS
- An exponential growth phase where the daily emergence was highest between 5 and 10 DAS
- A regression phase where the emergence gradually dropped between 10 and 14 DAS
- A seedling mortality phase from 15 DAS (Fig. 1 and 2)

All the treatments reached a maximum of emerged seedlings 12-14 DAS. Beyond 14-15 DAS, the number of emerged seedlings dropped (Fig. 1, 2). In terms of treatments containing *T. harzianum* isolates, the emergence speed and the number of surviving seedlings were often greater than in the treatments without *T. harzianum*. In addition, the seedling mortality phase was far shorter than in the control treatments.

The *T. harzianum* isolate from Burkina Faso provided a higher seedling emergence speed than the isolate from South Africa (Fig. 1, 2). Depending on the treatment methods used, seed coating with *T. harzianum* had a greater effect on the speed of seedling emergence than spraying the seedbed with a conidial suspension of *T. harzianum* (Fig. 1, 2).

Effect of *T. harzianum* on seedling growth: The growth parameters of seedlings (surviving seedlings, seedling length, seedling root length, number of leaves and fresh weight of seedlings), 30 days after sowing in the presence or not of *T. harzianum* are shown in Table 3. Coating the seeds and spraying the seedbeds with the conidial suspension of the two *T. harzianum* isolates significantly increased all these growth parameters compared to the controls, except for the number of leaves where the differences among treatments were not significant (Table 3). Seed coating was found to be more effective than seedbed spraying (Table 3). The *T. harzianum* isolate from Burkina Faso was more effective in promoting seedling emergence than the isolate from South Africa whatever the treatment method used (Table 3).

Protective effect of *T. harzianum* isolates on onion seedlings

Effect of pathogens on seedling emergence speed: Two onion pathogens, *F. oxysporum* and *F. solani* (two strains for each) were used as inoculums and mixed in the substrates where seeds were sown. Results of emergence of seedlings grown on these inoculated seedbeds from coated seeds and on sprayed seedbeds with *T. harzianum* isolates are shown in

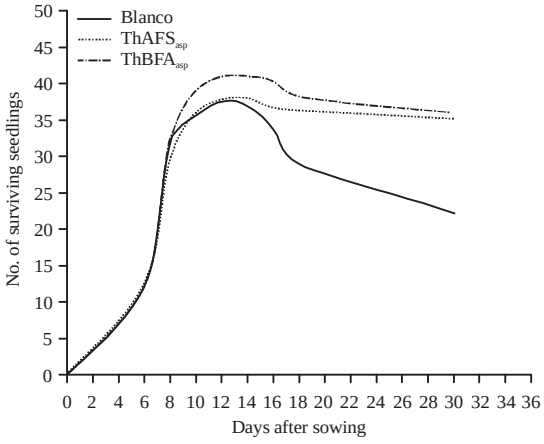

Fig. 1: Seedling emergence speed on sprayed substrates with *Trichoderma harzianum*

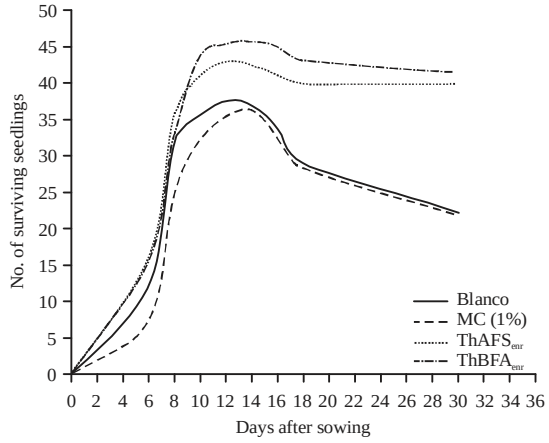

Fig. 2: Seedling emergence speed of coated seeds with *Trichoderma harzianum*

Fig. 3 and 4. The presence of each pathogen strain alone led to a sharp reduction in seedling emergence speed. The *F. oxysporum* strains were more active in this reduction than the *F. solani* strains (Fig. 3, 4). The seedling emergence ceiling was reached at 13 DAS for the control (38 emerged seedlings),

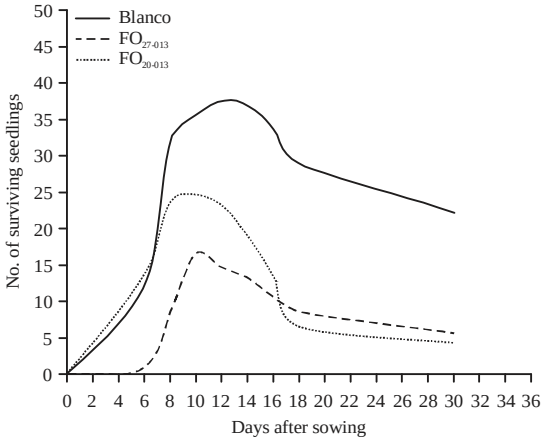

Fig. 3: Emergence of seedling grown on inoculated substrates with *Fusarium oxysporum*

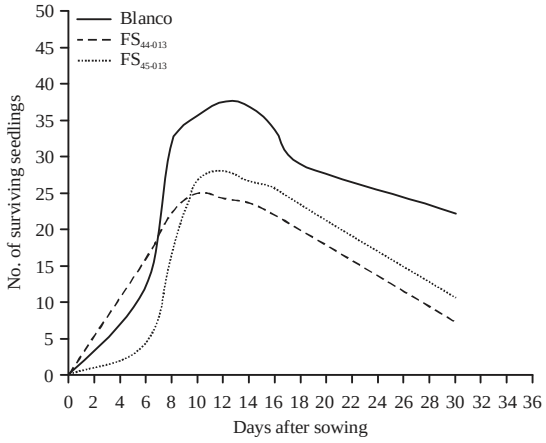

Fig. 4: Emergence of seedling grown on inoculated substrates with *Fusarium solani*

between 9 and 10 DAS with the *F. oxysporum* strains (17 and 25 emerged seedlings) (Fig. 3) and between 10 and 12 DAS with the *F. solani* strains (25 and 28 emerged seedlings) (Fig. 4). The number of emerged seedlings dropped significantly between the ceiling dates and 30 DAS. This drop was more pronounced with the *F. oxysporum* strains (Fig. 3). Among *F. oxysporum* strains, the action of FO_{27-013} on seedling emergence speed was more negative than the action of FO_{20-013} until 16 DAS. Beyond 16 DAS, the opposite effect occurred (Fig. 3). Among the *F. solani* strains, the same phenomenon occurred at the tenth DAS (Fig. 4).

Effect of *T. harzianum* on seedling damping-off: The effects of *T. harzianum* on the biocontrol of *F. oxysporum* and *Fusarium solani* are shown in Table 4 and 5. For the substrates inoculated with *F. oxysporum* strains, the general damping-off of seedlings (GDR) varied from 44.7-91.3% (Table 4) according to the treatments and for the substrates inoculated with *F. solani* strains it varied from 13.3-85.3% (Table 5). The substrates inoculated with the pathogens alone had the highest damping-off rates which were significantly different from those of the uninoculated substrates. The seedling damping-off due to *F. oxysporum* strains was slightly more severe than to *F. solani*. Among the *F. oxysporum* strains, Fo_{20-013} from Mogtédo was more pathogenic than Fo_{27-013} from Solenzo (Table 4) and among the *F. solani* strains Fs_{45-013} from Débé was the most pathogenic (Table 5). As in the case of seedbed spraying, the addition of *T. harzianum* isolates through seed coating significantly improved the number of emerged and surviving seedlings and reduced the GDR of the seedlings 30 DAS compared with the pathogen-alone treatment (Table 4, 5). The portion of damping-off due to pathogens action varied from -7.3-36.0% for the substrates inoculated with *F. oxysporum* and from -42-30% for those inoculated with *F. solani* (Table 4, 5). All the treatments with *T. harzianum* significantly reduced seedling damping-off caused by the

Table 3: Effect of *Trichoderma harzianum* on onion seedlings (in the absence of inoculated pathogens)

		Seedlings growth parameters				
Trichoderma harzianum inoculum	Codes	SN	SL (cm)	RL (cm)	NL	SW (g)
No *T. harzianum*	Blanco	22.3^{a}	20.4^{a}	4.2^{a}	2.9^{a}	0.1^{a}
	MC (1%)	21.8^{a}	19.0^{a}	3.2^{a}	2.9^{a}	0.1^{a}
T. harzianum in substrate spraying	$ThAFS_{asp}$	35.2^{b}	25.3^{b}	5.6^{b}	2.9^{a}	0.2^{b}
	$ThBFA_{asp}$	36.0^{bc}	27.3^{b}	6.2^{b}	3.0^{a}	0.2^{b}
T. harzianum in seed coating	$ThAFS_{enr}$	39.8^{bc}	33.4^{c}	12.4^{c}	3.0^{a}	0.4^{c}
	$ThBFA_{enr}$	41.5^{c}	33.6^{c}	12.8^{c}	2.9^{a}	0.4^{c}
Statistics	F-values	19.254	59.943	67.607	1.195	174.236
	p-values	0.000	0.000	0.000	0.335	0.000

Means followed by the same letter in each column are not significantly different at the 0.05 level using the Duncan range test, SN: Seedling number, SL: Seedling length, RL: Root length, NL: No. of leaves, SW: Seedling weight

pathogen action (Table 4, 5). For all the parameters evaluated, the efficiency of the *T. harzianum* treatment varied depending on the application method, isolate and pathogen. The *F. solani* strains were more sensitive to the action of *T. harzianum* than the *F. oxysporum* strains (Table 4, 5). Seed coating with *T. harzianum* isolates was more effective in reducing seedling damping-off due to pathogens than seedbed spraying with *T. harzianum* isolates. The *T. harzianum* isolate from Burkina Faso was more effective than the one from South Africa.

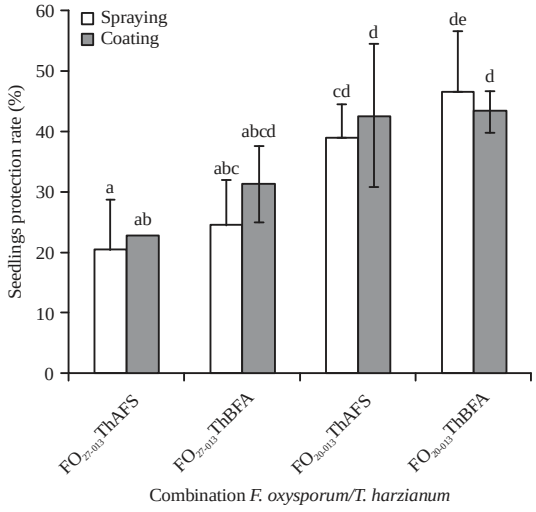

Fig. 5: Seedling protection by *Trichoderma harzianum* against *Fusarium oxysporum*. The bars affected with the same letter(s) in each series are not significantly different at the 0.05 level using the Duncan range test

Seedlings protection by *T. harzianum* isolates: The difference between seedling damping-off due to pathogens alone and when a *T. harzianum* treatment was present, considered as the rate of seedling protection by *T. harzianum,* is shown in Fig. 5 and 6, respectively for *F. oxysporum* and *F. solani*. Considering *F. oxysporum*, the *T. harzianum* treatment was more effective in protecting the seedlings against $Fo_{20\text{-}013}$ than $Fo_{27\text{-}013}$ but no significant difference between the modes of application of *T. harzianum* was observed (Fig. 5). Considering *F. solani*, treatment with *T. harzianum* was more effective in protecting the seedlings against $Fs_{45\text{-}013}$ than $Fs_{44\text{-}014}$.

In general some differences of protection were observed depending on the mode of application but no clear tendency was observed (Fig. 5, 6).

Table 4: Effect of *Trichoderma harzianum* sprayed on the substrate or applied by seed coating, on the biocontrol of *Fusarium oxysporum* on onion seedlings

Trichoderma harzianum inoculum	Codes	No. of surviving seedlings	General damping-off rates (%)	Seedling damping-off due to pathogens (%)
No *T. harzianum*	Blanco	22.3^{cde}	55.3^{bcd}	00.0^{bcd}
	$Fo_{27\text{-}013}$ alone	5.7^{a}	88.7^{f}	33.3^{f}
	$Fo_{20\text{-}013}$ alone	4.3^{a}	91.3^{f}	36.0^{f}
T. harzianum in substrate	$Fo_{27\text{-}013}$ $ThAFS_{asp}$	16.0^{bc}	68.0^{de}	12.7^{de}
spraying	$Fo_{27\text{-}013}$ $ThBFA_{asp}$	18.0^{cd}	64.0^{cd}	8.7^{cd}
	$Fo_{20\text{-}013}$ $ThAFS_{asp}$	24.0^{de}	52.0^{bc}	-3.3^{bc}
	$Fo_{20\text{-}013}$ $ThBFA_{asp}$	27.7^{e}	44.7^{B}	-10.7^{b}
T. harzianum in seed	$Fo_{27\text{-}013}$ $ThAFS_{enr}$	17.0^{bcd}	66.0^{cde}	10.7^{cde}
coating	$Fo_{27\text{-}013}$ $ThBFA_{enr}$	21.3^{cde}	57.3^{bcd}	2.0^{bcd}
	$Fo_{20\text{-}013}$ $ThAFS_{enr}$	25.7^{e}	48.7^{b}	-6.7^{b}
	$Fo_{20\text{-}013}$ $ThBFA_{enr}$	26.0^{e}	48.0^{b}	-7.3^{b}
Statistics	F-values	19.198	19.198	19.289
	p-values	0.000	0.000	0.000

Means followed by the same letter in each column are not significantly different at the 0.05 level using the Duncan range test

Table 5: Effect of *Trichoderma harzianum*, sprayed on the substrate or applied by seed coating, on the biocontrol of *Fusarium solani* on onion seedlings

Trichoderma harzianum inoculum	Codes	No. of surviving seedlings	General damping-off rates (%)	Seedling damping-off due to pathogens (%)
No *T. harzianum*	Blanco	22.3^{cde}	55.3^{bcd}	00.0^{bcd}
	$Fs_{44\text{-}014}$ alone	10.7^{ab}	78.7^{ef}	23.3^{ef}
	$Fs_{45\text{-}013}$ alone	7.3^{a}	85.3^{f}	30.0^{f}
T. harzianum in substrate	$Fs_{44\text{-}014}$ $ThAFS_{asp}$	27.0^{d}	46.0^{b}	-9.3^{b}
spraying	$Fs_{44\text{-}014}$ $ThBFA_{asp}$	27.3^{d}	45.3^{b}	-10.0^{b}
	$Fs_{45\text{-}013}$ $ThAFS_{asp}$	37.3^{f}	25.3^{a}	-30.0^{a}
	$Fs_{45\text{-}013}$ $ThBFA_{asp}$	36.7^{f}	26.7^{a}	-28.7^{a}
T. harzianum in seed	$Fs_{44\text{-}014}$ $ThAFS_{enr}$	21.3^{cde}	57.3^{bcd}	2.0^{bcd}
coating	$Fs_{44\text{-}014}$ $ThBFA_{enr}$	23.3^{cde}	53.3^{bcd}	-2.0^{bcd}
	$Fs_{45\text{-}013}$ $ThAFS_{enr}$	26.3^{e}	47.3^{b}	-8.0^{b}
	$Fs_{45\text{-}013}$ $ThBFA_{enr}$	43.3^{f}	13.3^{a}	-42.0^{a}
Statistics	F-values	19.198	19.198	19.289
	p-values	0.000	0.000	0.000

Means followed by the same letter in each column are not significantly different at the 0.05 level using the Duncan range test

Effect of *T. harzianum* on seedling growth parameters in the presence of pathogens: The application of *T. harzianum* treatment significantly improved the number of emerged seedlings but also the seedling and root length compared to the treatments with all tested pathogens alone.

In the trays inoculated with *F. oxysporum* strains, the application of *T. harzianum* by seed coating didn't improved significantly the number of formed leaves compared to strains alone. However, by spraying, the treatment of *T. harzianum* significantly increased the number of leaves (Table 6). In the trays inoculated with *F. solani* strains, the application of *T. harzianum* by the two modes significantly improved the number of leaves at the level of one strain (FS_{44-014}) (Table 7).

Considering the weight of seedlings, the application of *T. harzianum* by seed coating significantly increased it compared to the others treatments. No significant difference was observed by substrate spraying (Table 6, 7).

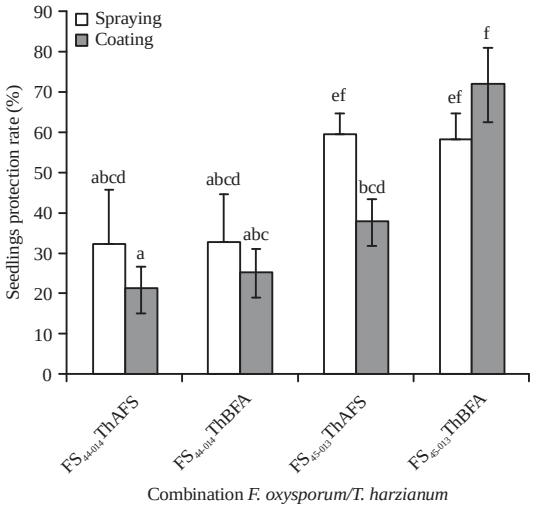

Fig. 6: Seedling protection by *Trichoderma harzianum* against *Fusarium solani*. The bars affected with the same letter(s) in each series are not significantly different at the 0.05 level using the Duncan range test

DISCUSSION

Fusarium oxysporum and *F. solani* are deeply implicated in seed rot and seedling damping-off in onion[35,36]. *Fusarium oxysporum* is also reported to be responsible for onion basal rot in many production areas[35]. These fungi were the main pathogens found in the onion seed samples used in Burkina Faso according to a previous study. Some species of the genus *Trichoderma* are commonly used as biocontrol agents against plant pathogenic fungi and some isolates of these species produce metabolites that improve plant growth[37]. Using *T. harzianum* in onion-growing areas might greatly reduce seedling damping-off, increase seedling growth and vigor, prevent diseases appearing in fields and prevent the wastage of seeds. Such biological control method could also be cheap, locally available and environmentally friendly for resource-poor famers in Burkina Faso. The overall aim of this study was to evaluate the efficiency of two *T. harzianum* isolates in controlling seedling damping-off and promoting seedling growth.

Growth promotion properties of *T. harzianum*: Spraying substrates in onion nurseries with a conidial suspension of *T. harzianum* and coating seeds with *T. harzianum*

Table 6: Effect of *Trichoderma harzianum* on onion seedlings (in the presence of *Fusarium oxysporum*)

		Seedling growth parameters				
Trichoderma harzianum inoculum	Codes	SN	SL (cm)	RL (cm)	NL	SW (g)
No. *T. harzianum*	Blanco	22.3^{de}	20.4^{fg}	4.2^{f}	2.9^{de}	0.10^{a}
	Fo_{27-013} alone	05.7^{a}	08.5^{ab}	0.4^{a}	1.9^{a}	0.02^{a}
	Fo_{20-013} alone	04.3^{a}	06.7^{a}	0.8^{ab}	2.5^{abcde}	0.20^{a}
T. harzianum in substrate spraying	Fo_{27-013} $ThAFS_{asp}$	16.0^{ab}	14.0^{cd}	3.1^{de}	2.1^{ab}	0.09^{a}
	Fo_{27-013} $ThBFA_{asp}$	18.0^{cd}	12.6^{c}	2.4^{cd}	2.1^{ab}	0.05^{a}
	Fo_{20-013} $ThAFS_{asp}$	24.0^{de}	15.6^{d}	3.1^{de}	2.8^{cde}	0.06^{a}
	Fo_{20-013} $ThBFA_{asp}$	27.7^{e}	16.5^{de}	2.4^{cd}	2.7^{bcde}	0.07^{a}
T. harzianum in seed coating	Fo_{27-013} $ThAFS_{enr}$	17.0^{cd}	21.9^{fgh}	3.2^{de}	2.0^{a}	0.72^{de}
	Fo_{27-013} $ThBFA_{enr}$	21.3^{cde}	24.1^{h}	3.4^{def}	2.0^{a}	0.59^{bcde}
	Fo_{20-013} $ThAFS_{enr}$	25.7^{e}	23.4^{gh}	3.5^{ef}	2.2^{abc}	0.3^{abc}
	Fo_{20-013} $ThBFA_{enr}$	26.0^{e}	20.7^{fg}	3.5^{ef}	2.2^{abc}	0.23^{ab}
Statistics	F-values	19.339	48.390	12.432	3.619	4.589
	p-values	0.000	0.000	0.000	0.000	0.000

Means followed by the same letter in each column are not significantly different at the 0.05 level using the Duncan range test, SN: Seedling number, SL: Seedling length, RL: Root length, NL: No. of leaves, SW: Seedling weight

Table 7: Effect of *Trichoderma harzianum* on onion seedlings (in the presence of *Fusarium solani*)

		Seedling growth parameters				
Trichoderma harzianum inoculum	Codes	SN	SL (cm)	RL (cm)	NL	SW (g)
No *T. harzianum*	Blanco	22.3^{de}	20.4^{fg}	4.2^{f}	2.9^{de}	0.10^{a}
	Fs_{44-014} alone	10.7^{ab}	09.5^{ab}	1.5^{bc}	2.1^{ab}	0.17^{a}
	Fs_{45-013} alone	07.3^{a}	09.7^{b}	1.9^{bc}	3.0^{e}	0.31^{abc}
T. harzianum in substrate spraying	Fs_{44-014} $ThAFS_{asp}$	27.0^{e}	19.1^{ef}	3.1^{de}	2.4^{abcde}	0.08^{a}
	Fs_{44-014} $ThBFA_{asp}$	27.3^{e}	20.7^{fg}	3.6^{ef}	2.7^{bcde}	0.11^{a}
	Fs_{45-013} $ThAFS_{asp}$	37.3^{f}	23.1^{gh}	3.9^{ef}	2.9^{de}	0.18^{a}
	Fs_{45-013} $ThBFA_{asp}$	36.7^{f}	21.7^{fgh}	3.0^{de}	3.0^{e}	0.16^{a}
T. harzianum in seed coating	Fs_{44-014} $ThAFS_{enr}$	21.3^{cde}	23.9^{h}	4.3^{f}	2.5^{abcde}	0.58^{bcde}
	Fs_{44-014} $ThBFA_{enr}$	23.3^{cde}	22.2^{fgh}	4.4^{f}	2.3^{abcd}	0.82^{e}
	Fs_{45-013} $ThAFS_{enr}$	26.3^{e}	29.2^{i}	4.0^{ef}	3.0^{e}	0.40^{abcd}
	Fs_{45-013} $ThBFA_{enr}$	43.3^{f}	28.9^{i}	4.3^{f}	3.0^{e}	0.64^{cde}
Statistics	F-values	19.339	48.390	12.432	3.619	4.589
	p-values	0.000	0.000	0.000	0.000	0.000

Means followed by the same letter in each column are not significantly different at the 0.05 level using the Duncan range test, SN: Seedling number, SL: Seedling length, RL: Root length, NL: No. of leaves, SW: Seedling weight

accelerated seedling emergence speeds and significantly increased seedling emergence rates, seedling length and weight and root length compared to untreated substrate and uncoated seeds. These results are in accordance with a lot of studies, such as Gasoni *et al.*[38], who reported an improvement in the seedling number and fresh weight of table beet by sowing beet seeds coated with a *T. harzianum* isolate. Bioplastic coatings containing spores of *T. harzianum*, significantly stimulated the germination of corn and canola seeds and enhanced their seedlings shoot and root lengths in relation to uncoated seeds[39]. Hoyos-Carvajal *et al.*[37] reported similar results for beans when using *T. harzianum*. Plant growth promotion by *Trichoderma* species was also reported in several studies. Contreras-Cornejo *et al.*[40] found that *T. virens* increased the lateral root formation and biomass production of *Arabidopsis*. The ability of some *Trichoderma* isolates to act as plant growth promoters is attributed to several mechanisms: The production of auxin-related compounds, such as indole-3-acetic acid, indole-3-acetaldehyde and indole-3-ethanol, that affect the growth parameters of the plants[40], conversion of insoluble phosphates into phosphate that is directly usable by plants[37], production of metabolites resulting in the synthesis of phytoalexins, PR proteins and other compounds in plants[41,42]. Some authors, however, reported that *Trichoderma* isolates and their enzymes can be very sensitive to environmental changes such as temperature and pH[43]. This needs to be taken into account in future trials in order to better work out the conditions for using both *T. harzianum* isolates in Burkina Faso. A comparison of the results of the coating and spraying using *T. harzianum* isolates indicated that seed coating was significantly more effective than substrate spraying. Butt and Copping[14] suggested that the efficiency of *Trichoderma* was related to the use shape. Therefore, if seed coating is to be promoted, the challenge is to experiment a cheap and available sticker under Burkina Faso conditions. Another possibility would be to create seed treatment centers where seeds are coated with *T. harzianum* isolates and sold to farmers.

Seedling protection by *T. harzianum*: The inoculation of seedbeds with *Fusarium* strains alone significantly modified emergence speed and reduced the number of emerged and surviving seedlings compared with the untreated substrates. That confirms the pathogenic action of the strains used in the study. Indeed, *Fusarium* species are reported to be causal agents of seed rot and seedling damping-off in several crops[35,44,45]. Dissanayake *et al.*[46] reported that shallot (*Allium fistulosum*) seeds, inoculated with strains of *F. oxysporum*, *F. solani* and *F. moniliforme*, lost their germinative power at rates 50-65%. The results of studies by Ozer *et al.*[47] showed that seed rot and seedling mortality caused by *Fusarium* species were due to an abundant secretion of polygalacturonase by the fungi. The polygalacturonase caused rapid damage in the seed tissues and was the main factor of seed death.

The addition of the two *T. harzianum* isolates through seed coating or seedbed spraying with conidial suspension greatly alleviated the damage caused by *Fusarium* species. Numerous examples of favorable effects of seed treatment with *T. harzianum* are reported. Carvalho *et al.*[25] showed that conidial suspension of two isolates of *T. harzianum* used in common bean seed treatment, significantly reduced the damping-off caused by *F. oxysporum* f. sp. *phaseoli* and enhanced seed germination. In a greenhouse experiment,

Dal Bello *et al*.[16] used coated wheat seeds with a solution of sodium carboxymethyl cellulose (CMC) and found that a isolate of *T. harzianum* significantly favored the plant stand, increased the percentage of emerging seedlings, the plant height and dry weight of wheat 3 weeks after sowing in substrate inoculated with *F. graminearum*. Rojo *et al*.[48] reported that coating seeds with conidial suspension of *T. harzianum* reduced root rot in peanuts caused by *F. solani*. This study is the first one revealing the positive effect of a strain of *T. harzianum*, native to onion crop in Burkina Faso, on onion growth and on *Fusarium* damping off, a major constraint in this area.

The mycoparasitic activity of *T. harzianum* against *Fusarium* species is usually attributed to a combination of successful nutrient and rhizosphere colonization competition, production of cell wall-degrading enzymes such as harzianic acid, alamethicins, tricholin, peptaibols, 6-penthyl-alpha-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and antibiosis[41,49-51]. Therefore, several *Trichoderma* isolates are being tested as alternatives to chemical fungicides. The use of *Trichoderma* for the biological control of plant pathogens, however, is not yet widespread.

The results of this study confirm the efficacy of both *T. harzianum* isolates in using both treatment methods. These two isolates can be used for onion seed coating or seedbed spraying in order to enhance seedling emergence and growth and to prevent seed rot and seedling damping-off caused by *Fusarium* species. Although seed coating exhibited sometimes the best results, they were not always significantly different from those obtained by spraying substrates with conidial suspension. Given the cost and availability of stickers for coating seeds with *T. harzianum* conidia, the direct spraying of seedbeds with conidial suspension is probably a more accessible method for producers in Burkina Faso. An issue that will arise with this method is finding a simple and efficient way for mass production of conidia by farmers themselves. In this respect, agro-industrial by-products, accessible for resource-poor farmers could be tested as potential carrier material for the mass production of *T. harzianum*. Seed coating using available stickers such as clay powder could also been explored. Another option for the appropriate use of *T. harzianum* in field conditions would be to produce enriched compost with the fungus conidia.

CONCLUSION

This study concluded that the two isolates of *T. harzianum* originating from Burkina Faso and South Africa showed strong properties in onion growth promotion and seedling protection against damping-off caused by *F. oxysporum* and *F. solani*. The use of an indigenous isolate of *T. harzianum* originated from Burkina Faso could be a novel biocontrol strategy against the damping-off of onion seeds in an environmentally sustainable way. Given these findings, it is important to work out a simple and accessible method of mass production of the indigenous isolate of *T. harzianum* by farmers and to conduct experimental assays under different agro-ecological conditions of Burkina Faso.

SIGNIFICANCE STATEMENTS

- *Trichoderma harzianum* native to Burkina Faso was isolated from a soil in the rhizosphere of onion (*Allium cepa* L.) crop
- This isolate significantly improved the germination and growth of onion on sterilized substrate under greenhouse conditions
- This isolate also alleviated the damage caused on onion by *Fusarium oxysporum* and *F. solani*, major pathogens of onion in tropical areas
- The onion growth promotion and its protection against *Fusarium* damping-off were higher when sowing *T. harzianum* coated seeds than spraying seedbed substrate and sowing untreated seeds
- The use of native *T. harzianum* can be a novel biocontrol strategy against the damping off of onion in an environmentally sustainable way, adapted to West African ecosystems

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