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Original article

Fungicide Tolerance and Effect of Environmental Conditions on Growth of *Trichoderma* spp. with Antagonistic Activity Against *Sclerotinia sclerotiorum* Causing White Mold of Common Bean (*Phaseolus vulgaris*)

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Abstract

The present study was conducted to evaluate *in vitro* compatibility of commonly used agrochemicals as well as the effect of temperature, pH and salt on the growth of six *Trichoderma* spp. with antagonistic activity against *S. sclerotiorum* responsible for white mold of common bean. The results revealed that in dual culture, the mycelial growth inhibition of *S. sclerotiorum* ranged from 83.4 to 87.4 %. The highest inhibition (87.4 %) was obtained with isolate *T. erinaceum* It-58, while the lowest inhibition (83.4 %) was caused by *T. koningiopsis* It-21. Except *T. asperellum* It-13, antagonistic fungi were able to fully colonized pathogen in five days reaching class I antagonism according to Bell scale. The maximum inhibition percentage of volatile (54.07 %) and non-volatile compounds (68.89 %) on pathogen was respectively caused by *T. asperellum* It-13 and *T. harzianum* P-11. Fungicides affect the growth of *Trichoderma* differently. No growth was observed while testing compatibility of *T. asperellum* It-13 and *T. erinaceum* It-58 with Mancozeb as well as *T. asperellum* It-13 and *T. afroharzianum* P-8 with Methyl thiophanate illustrating the absence of compatibility. The excellent growth rate of *Trichoderma* was found at temperature range of 25–30°C and pH range 4.5-5.5. Apart from *T. asperellum* It-13, all the isolates were able to grow at NaCl concentrations up to 1000 µM and were identified as superior salt-tolerant isolates.

Keywords: Antagonistic, S. sclerotiorum, Trichoderma, Biological control, Fungicide tolerance.

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INTRODUCTION

White mold of common bean (*Phaseolus vulgaris* L.) caused by the fungal pathogen, *Sclerotinia sclerotiorum* is a devastating disease that limits yield potential (Singh and Schwartz, 2010) and reduces seed and pod quality worldwide. With increase in the irrigated area cultivated with common bean, white mold has become the disease with highest economic importance, being able to cause 100% of losses in its production (Viera et al., 2010). The search for alternative control strategies as biocontrol, has increased in the last decades. The use of antagonistic microorganisms against plant pathogens has been considered more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). Among various antagonistic fungi used for the management of plant diseases, *Trichoderma* spp. have been widely studied as potential biocontrol agents for controlling many plant pathogens (Agrios, 2005; Mukherjee et al., 2012). Genus *Trichoderma* has helped to sustain the agricultural yields naturally, as a multifunctional agent (Hamed et al., 2015; Triveni *et al*., 2015). *Trichoderma* spp. are free-living filamentous fungi that are common in soil and root ecosystems. This ubiquitous filamentous fungus, with high survival rate, reproductive ability, nutrient use and capacity to promote plant growth through diverse mechanisms, has several species that are effective biological control agents against plant pathogens (Zhang et al., 2013; Brito et al., 2014, Abo-Elyousr et al., 2014). Typically, the antifungal properties of *Trichoderma* are attributed to their ability to produce volatile and non-volatile inhibitory compounds (Hua et al., 2014), or/and hydrolytic enzymes (Parmar et al., 2015) or their ability to induce host resistance to suppress disease development (Ting, 2014).

However, when planning the application of antagonistic *Trichoderma* strains for the purpose of biological control, it is important to consider various parameters affecting their growth (Zehra et al., 2017). Several abiotic factors such as temperature, water relations, salt, pH and even the pesticides have been already reported to deteriorate the antagonistic properties of *Trichoderma* species against the plant pathogenic fungi (Dluzniewska 2003; Zehra et al., 2017). *Trichoderma* strains are of great importance as biocontrol strains and should have better stress tolerance level than the plant pathogens against which they are to be used for biological control (Kredics et al., 2004). It becomes therefore important to study the degree of influence of these parameters on the growth of *Trichoderma* before full utilization of as biocontrol.

The objective of the present investigation was aimed to evaluate the *in vitro* biocontrol efficacy of *Trichoderma* isolates against *S. sclerotiorum* and effect of environmental factors and chemical fungicide on the growth of antagonists.

Material and Methods

Isolation of Trichoderma spp.

Trichoderma spp. were isolated from rhizospheric soil samples of cultivated bean plants collected from Soa, Center region, Cameroon by serial dilution technique on PDA and incubated at 28 °C for 4–6 days. Morphologically distinct colonies were selected and purified following sub-culturing. Six different *Trichoderma* isolates were identified up to species level on the basis of morphological and cultural characteristics by using the key of Rifai (1969), Samuels et al. (1994), Samuels (2006) and an online interactive key Samuels et al. (2004) at http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm. The identified isolates were *T. asperellum* It-13, *T. koningiopsis* It-21, *T. erinaceum* It-58, *T. gamsii* It-62, *T. afroharzianum* P-8 and *T. harzianum* P-11. All these *Trichoderma* spp. were used for further studies.

Isolation of S. sclerotiorum

Tissues of snap bean showing white mold disease symptom were obtained from the University of Agricultural Sciences and Medicinal Veterinary of Bucharest (USAMV) and used for isolation of the plant pathogen *S. sclerotiorum.* For isolation, infected host tissue were washed under tap water and cut out in small pieces (5-10 mm^2) from the leading edges of lesion. The pieces were surface-sterilized by dipping in 1% sodium hypochlorite solution for 5 min, and then rinsed three times with sterile distilled water, using dry out blotter for 3 min. Tissues were aseptically transferred into potato dextrose agar (PDA) plates. The plates were incubated at 28 °C. Mycelial emerging from tissues were transferred to the Petri dishes containing PDA to obtain pure cultures of the *S. sclerotiorum*. Specific virulence of the isolated *Sclerotinia sclerotiorum* was determined.

In vitro efficacy of Trichoderma spp. on growth of S. sclerotiorum

Dual culture experiment

Antagonistic efficacy of *Trichoderma* spp. was tested against the isolated pathogenic fungus *Sclerotinia sclerotium* by dual culture (Morton and Stroube, 1955). *Trichoderma* spp. and test fungus were inoculated 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 °C for 5 days. Monoculture plates of both served as control. Index of antagonism as percent growth inhibition of different antagonist versus plant pathogen was determined by following the method of Watanabe (1984). The degree of antagonism between each of the *Trichoderma* species and the test pathogen in dual culture was scored based on Bell scale, varying from 1-5 in which: class I = *Trichoderma* completely overgrow the pathogens (100% over growth); class II = *Trichoderma* overgrow at least two thirds of the pathogens (75% over growth); class III = *Trichoderma* colonizes on one half of the pathogens (50% over growth); class IV = *Trichoderma* and the pathogens contact point after inoculation, and class V = Pathogens overgrow bioagent *Trichoderma* (Bell et al.,1982).

Effect of volatile and non-volatile inhibitory compounds

The effect of the volatile metabolites produced by *Trichoderma* on mycelial growth was determined by the method of Dennis and Webster (1971). The antagonistic fungi were centrally inoculated by placing 5 mm diameter mycelia disc taken from 3 days old culture on the PDA plate and then incubated at 28 °C for 2 days. The top of each Petri dish was replaced with bottom of the PDA plate centrally inoculated with the pathogens. Two plates were sealed together with paraffin tape and further incubated at 28 °C. As for control, instead of *Trichoderma* spp., a 5 mm diameter of sterile PDA medium was used, being placed in plate. Three replications were employed for each treatment. Colony diameter of the pathogen was measured 72 h after incubation and the inhibition of mycelial growth determined.

The effect of the non-volatile metabolites produced by the antagonistic effective *Trichoderma* isolates on mycelial growth was determined by the method of Dennis and Webster (1971). Each antagonistic isolate was grown on a sterile cellophane disc laying on PDA in 9 cm Petri dishes for 48 h, then the cellophane with the mycelium was removed and in the same position where the mycelium was grown, a mycelia 5 mm diameter of the pathogens was inoculated. Three replications were employed for each treatment. Radial growth of the pathogen colonies was determined after and 6 days and compared with those of the pathogen grown on PDA without metabolites (control).

Fungicidal tolerance

The *in vitro* comparative toxicity of fungicides on the growth of *Trichoderma* was evaluated by poisoned food technique (Nene and Thapliyal, 1993). Fungicides viz Dithane M-45 (80% Mancozeb), Merpan-80 WDG (80% Captan), Topsin-70 WDG (70% Methyl thiophanate) were used. Stock solutions of chemicals were prepared by dissolving the required quantities of each into sterile distilled water. Increasing concentrations (½×recommended dose, recommended dose and 2×recommended dose) were then prepared and incorporated into the PDA culture medium kept molten at 50 °C and were mixed thoroughly by gentle shaking. The mixture was then poured into Petri plates. PDA plates without any added compounds served as controls. After solidification. Each plate was centrally inoculated with 5 mm inoculum disk from 6 day old culture of *Trichoderma* isolates. The inoculated dishes were incubated at 28 ºC. The observation on radial growth of the colony (mm) of the isolates in the Petri dishes treated with different fungicides at different doses was recorded. Three replicates were maintained for each treatment including one set of control separately for different doses. Per cent growth inhibition over the control was calculated.

Effect of temperature on the growth of Trichoderma spp.

The effect of temperature on the linear growth of *Trichoderma* was studied on PDA. From the periphery of 5 day old cultures of different isolates, mycelial discs were taken out by a cork borer (5

mm diameter) and placed at the center of the PDA plates. Plates were randomly incubated at different temperatures including 15; 20; 25; 30; 35 and 40 °C with three plates (replicates) per each isolate. Radial mycelial growth was compared and measured as the mean of two perpendicular diameters after 3 days and calculated in cm^2/day.

Effect of pH on growth of Trichoderma spp.

The effect of pH on the growth of different *Trichoderma* spp was tested by using PDA containing different pH levels. After preparation of the PDA, their suitable volumes were adjusted at pH 4.5, 5.5, 6.5, 7.5 and 8.5 using 1 N HCl or 1 N NaOH. The sterilized media of different pH levels was poured in the sterilized Petri plates and allowed to solidify. 5 mm diameter discs from the actively growing 5 day old cultures of different isolates were placed on the center of the Petri plates. The plates were incubated at 28 °C for 3 days after which the mycelia growth diameter was measured and calculated in cm^2/day.

Effect of NaCl concentration on growth of Trichoderma spp.

The effect of NaCl on the mycelia growth of *Trichoderma* spp was study on solid media according method describe by (Poosapati et al., 2014). From the periphery of 5 days old cultures of different isolates, mycelial discs were taken out by a cork borer (5 mm diameter) and placed at the center of the PDA medium supplemented with 250 µM; 500 µM; 750 µM and 1000 µM NaCl. Radial growth of different *Trichoderm*a was measured after 3 day of incubation at 28 ºC and the percentage of growth inhibition was calculated.

Statistical analysis

Statistical analyses of the data were performed using STATGRAPHICS Computer Software, version 5.0. The differences among treatments for all the studied parameters mentioned above were analyzed for statistical significance using analysis of variance (ANOVA). Treatments were compared by using Duncan's multiple range test at $P<0.05$ significance level.

Results

Antagonism in dual culture

Six native isolates of *Trichoderma* spp. were screened for their *in vitro* antagonism against isolated plant pathogen *S. sclerotiorum* under *in vitro* condition by dual culture technique (Table 1).

Table 1. In vitro effects of Trichoderma on the growth of S. sclerotiorum in dual culture

Isolates	*S. sclerotiorum* growth (mm)	Percent inhibition over control	Rating
T. asperellum It-13	12.33^{ab}	86.3^{ab}	Class II
T. koningiopsis It-21	14.67^{bc}	83.4^{bc}	Class I
T. erinaceum It-58	9.50^{a}	89.4^{a}	Class I
T. gamsii It-62	13.33^{d}	85.2^{d}	Class I
T. afroharzianum P-8	12.67^{c}	85.9^{c}	Class I
T. harzianum P-11	11.33^{ab}	87.4^{ab}	Class I
Control	90.00^{b}	0.00^{b}	-

*Means of three replications. In a column, means followed by a common letter are not significantly different at the P<0.05.

The results indicated that after five days incubation, antagonistic effect of *Trichoderma* spp. was in the range of 83.4 to 87.4%. *Trichoderma* species significantly inhibited (P< 0.05) the radial growth of *S. sclerotiorum* compared to control. The maximum inhibition of *S. sclerotiorum* was observed with *T. erinaceum* It-58 (89.4 %) followed respectively by *T. harzianum* P-11 (87.4 %) and *T. asperellum* It-13 (86.3 %). However, the minimum inhibition was observed with *T. koningiopsis* It-21 (83.4%).

According to Bell's scale, *Trichoderma* species were placed in class I and II. Indeed, all *Trichoderma* isolates tested except *T. asperellum* It-13, fully colonize the plant pathogen in five days reaching the Class I antagonism (Figure 1).

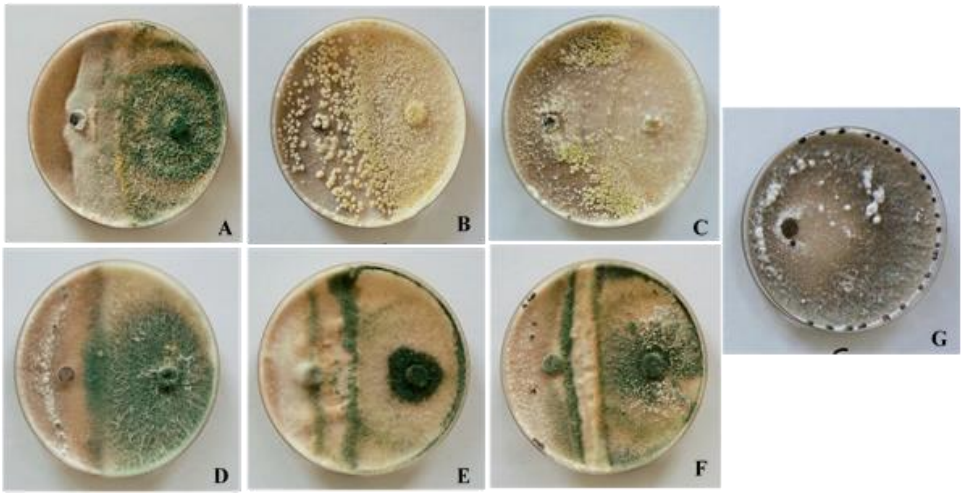

Figure 1. Mycoparasitism of Trichoderma isolates to phytopathogenic fungi S. sclerotiorum at 5 days incubation. (**A**) T. asperellum It-13 (B) T. koningiopsis It-21 (**C**) T. erinaceum It-58 (**D**) T. gamsii It-62 (**E**) T. afroharzianum P-8 (**F**) T. harzianum P-11 and (**G**) Control

Effects of volatile and non-volatile compounds

The effect of volatile and non-volatile compounds of *Trichoderma* on growth of *S. sclerotiorum* showed significant differences (P<0.05) compare to control and varied respectively from 29.63 % to 54.07 % and 46.30 to 68.89 % (Table 2).

Table 2. Inhibition percentage of volatile and non-volatile compounds from Trichoderma isolates on S. Sclerotiorum

Isolates	Inhibition Percentage (%)	
	Volatile	**Non-volatile**
T. asperellum It-13	54.07 ± 1.7^{ef}	46.30 ± 6.3^{cd}
T. koningiopsis It-21	50.74 ± 5.0^{de}	59.26 ± 1.3^{f}
T. erinaceum It-58	29.63 ± 5.5^{a}	51.48 ± 4.5^{de}
T. gamsii It-62	37.04 ± 6.5^{b}	55.67 ± 3.2^{ef}
T. afroharzianum P-8	40.74 ± 3.4^{bc}	62.22 ± 1.1^{ghi}
T. harzianum P-11	40.00 ± 5.1^{bc}	68.89 ± 1.1^{i}
Control	00.00 ± 0.0	00.00 ± 0.0

*Means of three replications. In a column, means followed by a common letter are not significantly different at P<0.05.

In case of volatile compounds, the minimum inhibition (29.63 %) was obtained with *T. erinaceum* It-58 whereas the maximum inhibition (54.07 %) was obtained with *T. asperellum* It-13. In the same way, for non-volatile compounds, the minimum inhibition (46.30 %) was obtained with non-volatile compounds of *T. asperellum* It-13 whereas the maximum inhibition (68.89 %) was obtained with *T. harzianum* P11.

Compatibility of Trichoderma spp. isolates with fungicides

The results of compatibility of *Trichoderma* spp. with fungicides indicated that all the three recommended fungicides affect the growth of *Trichoderma* at variable degree as shown in Figure 2.

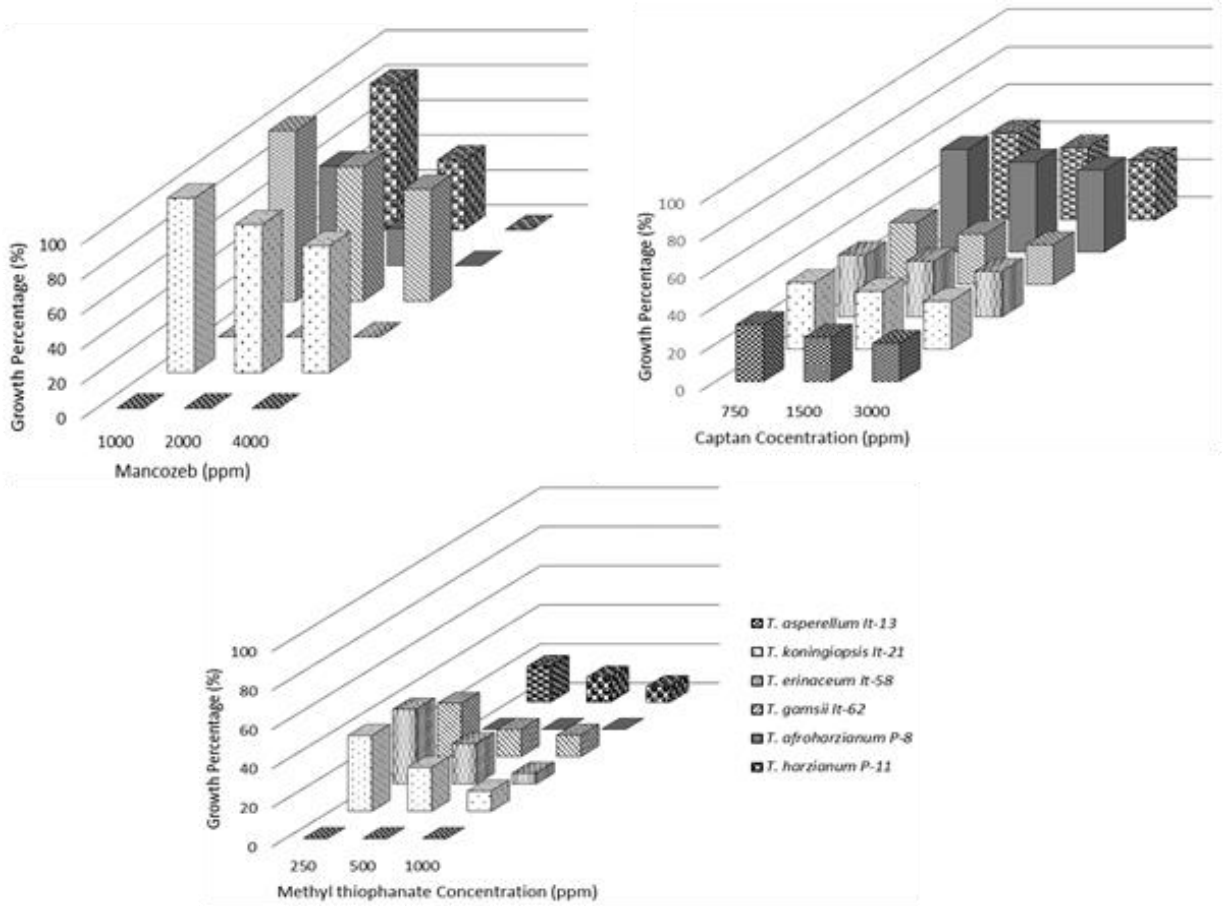

Figure 2. In vitro compatibility of three fungicides with Trichoderma spp. at various concentrations

All the tested fungi were able to develop in all tested concentrations of Captan therefore with various degree. Indeed, the lowest growth of 20.3 % was observed by *T. asperellum* It-13 at 3000 ppm whereas the highest growth of 54.6 % was recorded by *T. afroharzianum* P-8 at 750 ppm. At recommended dose of 1500 ppm *T. afroharzianum* P-8 show the highest growth of 47.9 % and the lowest development (23.6 %) was recorded by *T. asperellum* It-13.

Contrary to Captan, only *T. koningiopsis* It-21 and *T. gamsii* It-62 were able to growth in presence of all tested concentration of Mancozeb varying from 1000 to 4000 ppm. They growth of those fungi was varied respectively from 72.7 to 100 % and 64.2 to 97.6. However, no growth was recorded by *T. asperellum* It-13 and *T. erinaceum* It-58 at all tested concentration. In the same way, *T. afroharzianum* P-8 and *T. harzianum* P-11 were tolerant to Mancozeb up to 2000 ppm.

Concerning Methyl thiophanate, only *T. asperellum* It-21, *T. erinaceum* It-58, *T. gamsii* It-62 and *T. harzianum* P-11 were able to growth at tested concentration varying from 250 to 1000 ppm. The highest development (38.8 %) was recorded by *T. koningiopsis* It-21 followed by *T. erinaceum* It-58

(38.2%) and *T. gamsii* It-62 (27.3 %) at the lowest concentration tested 250 ppm. However no growth was observed while testing the fungicide on *T. asperellum* and *T. afroharzianum* P-8 at all concentration.

Effect of temperature

The present assay was undertaken to find out the optimum temperature for the growth of *Trichoderma* spp. by growing fungi at different temperatures on PDA. After 3 days of incubation the average radial growth was recorded and growth rate was determined. The results show that the growth rate of *Trichoderma* isolates varied from 0 to 27 cm^2/day according to the temperature and fungi tested (Figure 3).

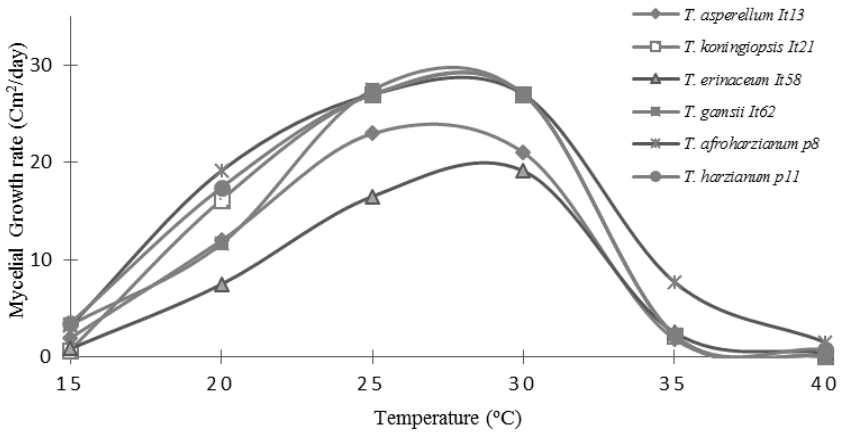

Figure 3. Effect of temperature on mycelial growth rate of different Trichoderma isolates

The maximum growth rate of all isolates tested was recorded at temperature range between 25-30˚C. At low temperature (15 °C), the growth was slow and all the fungi failed to full colonized Petri plate with the maximum growth rate of 3.5 cm^2/day recorded by *T. harzianum* P-11. Similar trend was also observed at high temperature (40 °C) where a maximum growth rate of 1.5 cm^2/day was observed with *T. afroharzianum* P-8. However, no growth was observed with *T. asperellum* It-13, *T. koningiopsis* It-21 and *T. gamsii* It-62 at this high temperature.

Effect of pH

According to Figure 4, mycelial growth rate varied from 10.5 to 42.9 cm^2/day according to the pH and isolate tested. All the fungi were able to develop at all pH evaluated and showed maximum growth rate at pH 5.5 except *T. koningiopsis* It-21 which showed a maximum growth rate at pH 5.5. The weakest mycelial growth rate (10.5 cm^2/day) was recorded at pH 8.5 with *T. erinaceum* It-58 whereas the highest mycelial growth rate (42.9 cm^2/day) was recorded at pH 5.5 with *T. harzianum* P-11. However, *T. afroharzianum* P-8 and *T. harzianum* P-11 were able to fully growth on Petri plate at all pH tested.

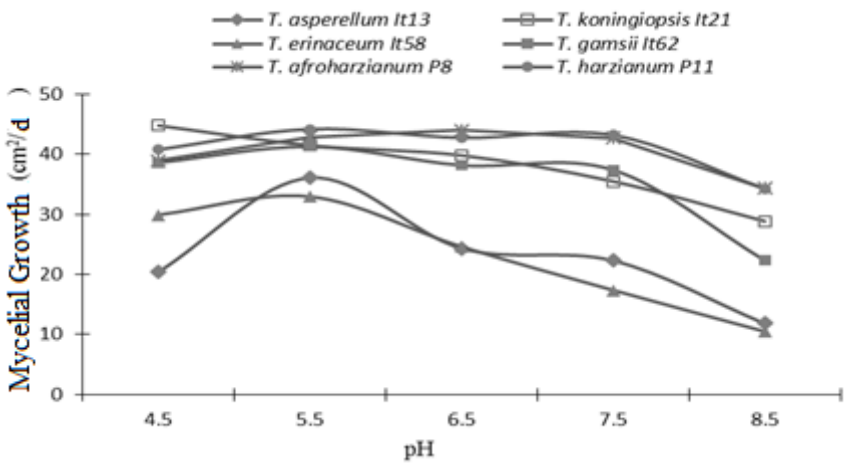

Figure 4. Effect of pH on mycelial growth rate of different Trichoderma isolates

Effect of NaCl

According to Table 3, all the six isolates of *Trichoderma* spp. showed a high range of NaCl tolerance and are able to develop at all tested concentration of salt.

Table 3. Effect of NaCl on mycelial growth (mm) of Trichoderma isolates.

Isolates	NaCl Concentration (µM)			
	250	500	750	1000
T. asperellum It-13	50 ± 3.9^{a}	33.8 ± 1.8^{def}	19.9 ± 0.64^{cd}	0 ± 0^{m}
T. koningiopsis It-21	49.7 ± 2^{a}	28.1 ± 3.7^{i}	19.7 ± 1.3^{fg}	18.3 ± 0.4^{h}
T. erinaceum It-58	49.7 ± 0a	23.2 ± 0.4^{gh}	17.9 ± 1.12^{def}	17 ± 0.6^{fg}
T. gamsii It-62	44.2 ± 0^{a}	21.9 ± 0.3^{cde}	18.4 ± 0.3^{bc}	15 ± 0.7^{efg}
T. afroharzianum P-8	60.2 ± 2.1^{c}	41.8 ± 1.5ij	14.5 ± 0.2^{ab}	11 ± 0.2^{l}
T. harzianum P-11	70.4 ± 0^{b}	41.7 ± 0.5^{j}	24.3 ± 0.5^{cde}	12 ± 0.1^{l}

*Mean of three replications. In a column, means followed by a common letter are not significantly different at P<0.05.

Indeed, at 1000 µM which was the maximal concentration of NaCl tested, the highest tolerance was observed by *T. koningiopsis* It-21 (18.3 mm) followed by *T. erinaceum* It-58 (17 mm). However, no growth was observed at 1000 µM concerning *T. asperellum* It-13. It was observed that at lowest concentration of NaCl 250 µM, the maximum growth (70.4 mm) was recoded with *T. harzianum* P-11 followed by *T. afroharzianum* P8 (60.2 mm) whereas the lowest growth (49,7 mm) was recorded simultaneously by *T. koningiopsis* It-21 and *T. erinaceum* It-58.

Discussion

Biocontrol is the most ecofriendly approach to the management of plant disease. The most important genus used as a biocontrol agent is *Trichoderma* (Spiegel and Chet, 1998) which has an

outstanding interaction with plant and plant pathogens (Mukherjee et al., 2012). The interactions include antagonism toward fungal pathogens, plant growth promotion, plant defense responses, and protection of plants from environmental stresses (Viterbo et al., 2010; Morath et al., 2012).

In this study, the results of dual culture experiment revealed that both the six isolates evaluated were effective in controlling colony growth of the tested pathogenic fungus S. sclerotiorum when compared to the control. The percentage of mycelial growth inhibition varied between from 83.4 to 87.4 %. The volatile and non-volatile metabolites also showed an effective performance on inhibiting the mycelial growth of the pathogen at various degrees with inhibition percentage range from 29.63 % to 54.07 % and from 46.30 to 68.89 %. One of the inhibition mechanism of mycelial growth of pathogens in dual culture may be due to competition for nutrients and space (Chet, 1987). Indeed it has been observed that our Trichoderma isolates grow rapidly and are able to invade the culture medium after 3 days of incubation. They have a faster growth rate than pathogen and a rapid and intense invasion. Similar findings have been reported by Sharma (2011) who showed that the growth of Trichoderma spp. is faster than that of the pathogen, so they colonize the nutrient medium faster and assimilate nutrients. The substantial functions associated with Trichoderma species are also their ability to produce a number of antibiotics as well as some cell wall degrading enzymes like β (1,3) glucanases, chitinases, proteases and sometimes cellulase able to degrade the mycelial walls of pathogens (El Katatny et al., 2001; De Castro et al., 2010). During the interaction between pathogen and some Trichoderma isolates, hyphal coiling and lysis of pathogens was observed. The coiling process is associated with internal mycoparasitism (Howell, 2003). Our results confirm the similar microscopic observations reported by Dubey et al (2007) and El-Hassan et al (2013). The same results were obtained with T. harzianum isolate-aloe capable of coiling on the S. sclerotiorum mycelium causing the disintegration of the cytoplasm of the pathogen (Zang et al., 2016). Belanger et al (1995) demonstrated that, during the interaction between T. viride and Botrytis cinerea, changes in the Botrytis membrane were detected 12 hours before physical contact between the two protagonists. They have attributed this observation to the effect of volatiles and non-volatiles diffusible compounds. Akrami et al (2011) and Saran et al (2013) have shown that Trichoderma species can act by antibiosis through the secretion of a wide range of metabolites that may be volatile in nature and non-volatile.

Studies reporting the effect of synthetic fungicides on the development of Trichoderma fungi have already been the subject of several studies: Bhale and Rajkonda (2015); Elshahawy et al (2016). In our study, experiments were conducted to determine the compatibility of biocontrol agents with 3 commercially effective chemicals in white mold of snap bean caused by S. sclerotiorum. The results obtained show that our isolates present varying levels of susceptibility from one fungicide to another and simultaneously from one concentration to another. These results are similar to those obtained by Bhale and Rajkonda (2015) who evaluated the compatibility of T. viride, T. harzianum, T. koningii, T.

pseudokoningii and T. virens against Mancozeb and Captan. They showed that these chemical fungicides were able to inhibit the radial growth of isolates to varying degrees. Similarly, Elshahawy et al (2016) assessed the compatibility of 10 isolates of Trichoderma spp against 7 chemical fungicides. They then showed that the different isolates of Trichoderma exhibit tolerance to 5 fungicides when the concentration varies from 50 to 800 ppm. Thus showing that the isolates were tolerant to all the thiophanate methyl doses tested but only tolerated up to 600 ppm Mancozeb. Moreover, in our study the isolate T. asperellum It-13 is not compatible with Mancozeb and thiophanate methyl, it is the same for T. erinaceum It-58 with Mancozeb. The T. afroharzianum P-8 isolate is incompatible with Methyl thiophanate. This incompatibility was shown in these isolates by no growth at all fungicide concentrations tested. The tolerance of some of our isolates to fungicides could be explained on the one hand by the ability of Trichoderma isolates to degrade chemical compounds when they are present in the medium at some concentrations. These chemical compounds are then assimilated to nutrients by the fungus (Hjeljord and Tronsmo, 1998). On the other hand, this compatibility could be explained as the result of a natural tolerance induced by the presence of ABC transporters present in Trichoderma (Harman et al., 2004). Indeed, Ruocco et al (2009) have shown the ability of Trichoderma to develop in the presence of high concentrations of synthetic or naturally occurring toxicants including its own antibiotic molecules depend on an effective detoxification mechanism which involves a complex system of transmembrane pumps. It is well known that Trichoderma genome contains genes encoding ABC transporters (ATP binding cassette transporters), which are members of a superfamily of proteins involved in the transport of xenobiotic substances out of the target cell (Chaparro et al., 2011).

In this study, the optimal growth rate of antagonistic Trichoderma isolates is range between 25-30˚C. T. asperellum It-13 and T. koningiopsis It-21 showed no growth at 40 ^{o}C, however at 15 ^{o}C, which was the lowest temperature tested, all isolates were developed. The results obtained are in perfect agreement with those obtained by Singh et al (2014) who have specified that each species of Trichoderma is characterized by a minimum and a maximum of temperature outside of which development is no longer possible. Studies conducted by Domingues et al (2015) on the effect of temperature on mycelial growth of four isolates of T. asperellum and a single isolate of T. asperelloides have shown that they can develop in temperatures ranging from 12 to 37 ^{o}C. They also recorded 27 ^{o}C as ideal temperature for the growth of these fungi. Similarly, Manoj et al (2017) have evaluated different temperature levels on the growth of 10 T. viride isolates and showed that the optimum growth temperature is between 25 and 30 ^{o}C. The heat stress tolerance observed when isolates are grown at 40 ^{o}C could be attributed on the one hand to the synthesis of heat shock proteins. Indeed, in many organisms, the synthesis of heat shock proteins (HSPs) appears as a heat tolerance mechanism (Schlesinger et al., 1982, Vierling, 1991). On the other hand, this tolerance could be justified by the increase in production and the accumulation of molecules such as trehalose, mannose and raffinose in the cells and the culture medium. Indeed, studies conducted by Ruijter et al (2003) showed that mannose

makes up 10 to 15% of the mycelial dry weight of filamentous fungi and intervenes in abiotic stress resistance such as extreme temperatures. Pedreschi et al (1997) observed an increase in trehalose accumulation in T. harzianum conidia when they were exposed to a heat shock of 40 °C for 90 minutes. Thus, the role of these sugars in the survival and stabilization of cellular structures and proteins under heat stress conditions in fungi of the genus Trichoderma has already been established (Ruijter et al., 2003; Poosapati et al., 2014).

The pH of the medium appears to be one of the most important parameters affecting the growth of Trichoderma fungi. It influences the mineral availability in these fungi as well as the rates of metabolic reactions and enzymatic activity (Zehra et al., 2017). In our study we have shown that Trichoderma isolates are able to grow in a pH range of 4.5 to 8.5 with optima growth between 4.5 and 5.5. This pH interval is then made up of optimal values for mycelial growth. The results obtained corroborate with those of Zehra et al (2017) who have shown that the pH optimum of species such as T. harzianum, T. viride, T. asperellum, T. koningiopsis is between pH 4 and 6. These results are further supported by studies conducted by Kredics et al (2003) who showed that Trichoderma species were able to grow in a wide pH range from 2 to 6 with an optimum of 4. For their growth, fungi of the genus Trichoderma have more affinity for soils with acidic pH containing a large amount of organic matter (Upadhyay and Rai, 1979). It is indeed important to collect information on the effects of pH on mycelial growth of the antagonist agent because these are related to the activity of the extracellular enzymes produced, especially since the latter seem to be involved in the competitions for nutrients and mycoparasitism in fungi of the genus Trichoderma.

Salt stress is one of the abiotic factors that significantly impacts the soil microbial ecosystem and thus limits crop productivity (Poosapati et al., 2014). Soil salinity is an important environmental factor that limits the capacity of Trichoderma isolates and therefore plays a prominent role in the microbial selection process (Borneman et al., 1996). In our study, all Trichoderma isolates tested except T. asperellum It-21 were able to grow at all NaCl concentrations tested. These are then able to withstand NaCl concentrations of up to 1M. These results are in accordance with those obtained by Zehra et al (2017), who evaluated the effect of different environmental conditions on the growth of four Trichoderma isolates viz T. harzianum, T. viride, T. hamatum and T. asperellum and showed that these isolates were able to tolerate NaCl concentrations up to 1000 µM. However, in our case, no growth was observed with T. asperellum It-13. The development of microorganisms is closely related to the activity of water due to the influence of the osmotic pressure exerted by the medium on the transmembrane exchanges within the cells. The tolerance to salt stress exhibited by our isolates could then be justified by the fact that in the presence of high concentration of salts, fungi of the genus Trichoderma develop extrusion systems (efflux pump) allowing them to maintain an intracellular level sodium concentration below the toxic concentration for the cell (Gunde-Cimerman et al., 2009). On the other hand, one of the

physiological adjustments in microorganisms in case of reduction of water activity in the medium is the accumulation of specific intracellular substances, caused by an increase in synthetic metabolic pathways (Soliman et al., 1994). The substances that are accumulated are neutral osmolites that can act as osmoprotective agents (Anthony, 1998).

Conclusion

The antagonistic nature of *Trichoderma* species against pathogenic fungi *S. sclerotiorum*, effect of environmental stresses on growth of these antagonists and compatibility to fungicides were evaluated under *in vitro* conditions. The results of *in vitro* dual confrontation and antibiosis, indicated that our six isolates of *Trichoderma* have potential as biocontrol agents against *S. sclerotiorum* responsible of white mold of snap bean (*Phaseolus vulgaris*). It was observed that growth of pathogenic fungi was reduced with respect to radial growth. Results on tolerance on environmental stresses shown that these antagonist are able to support various levels of temperature, pH, NaCl concentrations and chemical fungicide dose with varying degrees. Those antagonists could be included in more comprehensive future research of their antagonistic effects against *S. sclerotiorum* as well as the effect of different environmental stresses on antagonistic abilities of fungi with respect to secretion of extra cellular enzymes, antibiotics and competition related food and space.

Conflicts of interest

The authors have no conflicts of interest with any parties or individuals.

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