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Integrated Assessment of Phytostimulation and Biocontrol Potential of Endophytic *Trichoderma* spp Against Common Bean (*Phaseolus vulgaris* L.) Root Rot Fungi Complex in Centre Region, Cameroon

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ABSTRACT

This study aimed to assess the growth promotion and the in-vitro and greenhouse efficacy of Trichoderma endophytes from bean root against Fusarium oxysporum f.sp. phaseoli, Fusarium solani f.sp. phaseoli and Macrophomina phaseolina, causing root rot in Cameroonians bean (phaseolus vulgaris l.) fields. From twelve Trichoderma endophytes, Trichoderma polysporum T1, Trichoderma atroviridae T2, Trichoderma sp. T3 and Trichoderma harzianum T8 were selected upon in vitro antifungal screening (up to 88.2% growth inhibition). The selected bioagents could synthetise and release proteolytic enzymes and phosphate solubilization factors. T. harzianum T8 and T. polysporum T1 released the most active volatile and non-volatile antifungal metabolites, which together with conidia had either inhibitory or stimulating effects on bean seeds germination. In greenhouse, T. hazianum T8 (94.16%) and T. sp T3 (74.16%) enhanced seed germination velocity and percentage as well as shoot elongation (71.8 cm) more than other isolates both in absence and the presence of F. solani. A significant reduction in disease indexes was registred with the overall biocontrol agents, the maximum being recorded with Trichoderma sp. T3 (up to 50%). This further highlight the understanding on either the pathogenic nature of Trichoderma isolates or the improvement of bean resistance through direct and/or indirect action on causal pathogens.

Key words: *Phaseolus vulgaris L. F.oxysporum, F. solani, M. phaseolina, Trichoderma spp, biocontrol.*

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INTRODUCTION

The common bean (*P. vulgaris* L.) is the most important grain legumes for direct human consumption in the world. The fresh leaves, immature pods (green bean) and dry seeds of the crop provide 30% of the caloric and more than 50% of the dietary protein requirements of households in Latin America and in Africa[1,2] , especially in low-income socio-economic strata where protein is often rare or completely absent from diets[3]. In 2013, the global bean production was approximately 22. 806,139 metric tons of which 22 percent came from Africa[4]. In Cameroon, bean is intensively grown with an annual yield of approximately 153.000 kg/ha, a value that is 30 folds low as compared to the crop's potential yield (up to 4500 tons/ha)[1,5]. Besides, a 0.9% yield decline has been projected per annum[6]. The reason of such yield shortage has been attributed to intensive land use due to increasing demand for higher yields that has led to nutrient deficiency, in particular phosphorus (P). in fact, this element is a major plant growth-limiting nutrient despite its large quantity in soils probably due to its immobilisation in the form of insoluble phosphates[7].

Elsewhere, soilborne diseases are major constrains for many agricultural important crop worldwide, including common bean. They are often caused by diverse groups of fungi and related organisms among which species of *Fusarium, Rhizoctonia, Pythium, Sclerotium, Aphanomyces*[8]. These phytopathogens can each infect its host, causing a characteristic disease, or may, if occurring together, infect the host in any possible combination, resulting in disease complexes[9,10]. Generally, the pathogen (s) attack (s) the roots parts and impair the proper functioning of the plant in water and nutrients uptake[9]. The observable symptoms include wilting, leaf fall, and death of branches and limbs and in severe cases death of the whole plant. Regarding this, an increasing impact of common bean root-rot devastating complex has been identified in central Africa this last decate[11]. Moreover, recent studies conducted in Cameroon in lowlands have let to the identification of this disease[12, 10]. But so far, reports on control strategies are still fragmentary.

However, crop rotation, soil conditioning, the use of resistant cultivars, fungicides and fertilizers are the common strategies used for the management of such constrains in agriculture. The most effective method in preventing root rots being seed coating with chemical fungicides[13]. Nevertheless, the worldwide trend towards environmentally safe methods for plant disease control in sustainable agriculture calls for reducing the use of synthetic chemicals since they have shown major drawbacks such as the lack of long-term efficacy due to the development of resistance by plant pathogens and the non-target environmental impacts. Therefore, there has been considerable interest in the discovery and application of environmentally-friendly fungicidal compounds. Thus an attempt has been made to develop biological control agents for sustainable agriculture[13, 14].

Biopreparations based on *Trichoderma* spp. as an effective biocontrol agent for plant diseases caused by soil-borne fungi has been reported both *in vitro* and under field conditions[15, 16, 13]. Its uses keeps growing in importance as the demand for more environmentally friendly management strategies for plant disease increases[8, 13]. Besides, it has been estimated that 90% of all antagonistic fungi used in plant protection belong to this genus[17]. Moreover, *Trichoderma* spp. has been used as Plant Growth Promoting Fungi (PGPF) due to its ability to produce siderophores, phosphate-solubilizing enzymes, and phytohormones[18]. Therefore, the present work was designed to screen the *in vitro* antagonistic effect of common bean endophytic *Trichoderma* spp. against major root pathogens prevailling in the Centre Region of Cameroon and their effects on seeds germination and seedling growth under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Source of phytopathogenic fungi isolates

The fungi pathogens used in this study were isolated from bean plants showing necrotic lesions on root and hypocotyls, collected during the 2013-2014 growing season from fields in some locations of centre Region Cameroon. (Table 1).

Small pieces of infected root and hypocotyls were surface sterilized (3% sodium hypochlorite for 5 minute), rinsed with sterile distilled water and blotted dry. The pieces were then placed on Potato Dextrose Agar (PDA) (Difco Laboratories, Detroit, MI, U.S.A.) medium supplemented with Chloramphenicol (500 mg per litre) and incubated (25°C) for 5 to 7 days. The developed fungal colonies were purified using hyphal tip techniques[19]. The fungi namely, *Fusarium solani*, *Fusarium oxysporum* and *Macrophomina phaseolina* were then identified according to their morphological and microscopic characteristics[20,21] and confirmed by the Laboratory of Soil Microboilogy of the Biotechnology Centre, University of Yaounde I. The pathogenicity test of the overall fungi was performed[9] (Data not shown). The re-isolation of the fungi from artificially infected roots was made as stated by Koch's rules[22].

Table 1. Sources and geographic origins of *Trichoderma* spp. used in this study

Isolate codes	Source	Geographic origin
Trichoderma sp. T1	*P. vulgaris* root	Makenene-Sud
Trichoderma sp. T2	*P. vulgaris* root	Ngoa-Ekelle
Trichoderma sp. T3	*P. vulgaris* root	Makenene-Centre
Trichoderma sp. T4	*P. vulgaris* root	Ndekalend
Trichoderma sp. T5	*P. vulgaris* root	Carrière
Trichoderma sp. T6	*P. vulgaris* root	Ndegetta
Trichoderma sp. T7	*P. vulgaris* root	Soa
Trichoderma sp. T8	*P. vulgaris* root	Ngoa-Ekelle
Trichoderma sp. T9	*P. vulgaris* hypocothyl	Ngoa-Ekelle
Trichoderma sp. T10	*P. vulgaris* hypocothyl	Bakongo
Trichoderma sp. T11	*P. vulgaris* hypocothyl	Carrière
Trichoderma sp. T12	*P. vulgaris* hypocothyl	Makenene-Est

Isolation and identification of biocontrol agents.

Isolation of *Trichoderma* spp was made from healthy and roted roots and hypocothyls of bean seedlings collected from seven locations (Stated above). Seedlings or roots of adult plants were washed thoroughly under running tap water and *Trichoderma* spp were isolated[19]. The isolated fungi colonies were identified at the genus level[20]. A representative sample made up of 12 *Trichoderma* isolates was selected for antifungal screening (Table 1**)** and the most potent was identified at species level based on the microscopic observations and colony characteristics following taxonomic keys[23,24]. Microscopic examination was carried out by mounting the cultures in lacto phenol cotton blue. Stock cultures of antagonists and pathogenic isolates were maintained on PDA slants and kept in a refrigerator (4°C) for further study.

***In vitro* mycoparasitic screening**

The mycoparasitic ability of selected *Trichoderma* isolates (Table 1) was perform using the dual culture technique as previously described by Holmes *et al*[25]. Briefly, 20 ml of sterilized melted PDA was plated in Petri plates (9 cm Φ) and allowed to solidify. Mycelial discs (6 mm Φ) from three day old cultures of both antagonistic fungi and the test pathogens were placed on a diagonal line opposite from each other at 5mm distance. The Petri plates either with pathogen or *Trichoderma* spp only inoculated at one end, served as control. The petri plates were then incubated at 28 ± 2°C. Three replications were made for each treatment.

The incubation period (days) after which two colonies came in contact (C) was recorded and the percentage growth inhibition was calculated. The biological control ability of each *Trichoderma* spp. was determined per interaction and per pathogen using the following formula[26]:

PBCI = M/Z.P

Where: PBCI (mm^{-1}) = Pakdaman's Biological Control Index; Z (in days) = period after inoculation till the fungal biological control agent fully grew over a pathogen colony;

M (in days) = period after inoculation till the fungal biological control agent fully grew on theplate and ; P (in mm) = the radial distance of pathogen colony growth between the edge of the inoculation disc and the marginal point of the colony located on the presumed diagonal line connecting centers of two discs in the plate.

***In vitro* inhibitory effect of extracellular metabolites releassed by the bioagents**

Inhibitory effect of volatile metabolites

The effect of volatile metabolites produced by the promising bioagents was assessed[27]. The antagonistic fungi were centrally inoculated by placing a one week old mycelia disc (6 mm Φ) on the PDA plate and incubated at 26±2°C for 2 days. The top of each Petri dish was then replaced with the bottom of a PDA plate centrally inoculated with the pathogen or with non inoculated PDA disk (Negative control). The so-combined Petri dishes were sealed together and further incubated at 26±2°C. After 2, 5 and 8 days of incubation, a linear growth of pathogenic fungi in all treatments was recorded. The decrease in percentage that occurred in linear growth of the pathogenic fungi was determined[28]

Reduction in linear growth = [(R1 - R2)/ R1] ×100

Where: R1= the radius of normal growth in control plates; R2= the radius of inhibited growth.

Inhibitory effect of extracellular non-volatile metabolites

The poisoned food technique was used to evaluate the effect of culture filtrate from *Trichoderma* sp on the growth of isolated root rot causing pathogens[29]. *Trichoderma* isolates were inoculated in 100 ml sterilized Potato Dextrose Broth (PDB) in 250 ml conical flasks and incubated at 25 ± 2°C on a rotary shaker (IKA-VIBRAX-VXR) at 150 rpm for 10 days. The mycelium was separated to culture filtrate by filtration through Whatman filter paper N°1. The culture filtrate was then sterilized by passing through a 0.45 then 0.22 µm millipore microbiological membranes (PALL Life sciences Acrodisc® PN 4602). PDA was amended with culture filtrate at 25%, poured in a Petri plate and inoculated with pathogen mycelial disk. The control Petri plate was amended with an equal amount of distilled water. The colony diameter of the pathogens was measured 2, 5 and 8 days after incubation**.** The percentage growth inhibition (GI) was determined using the following formula[30] with a modified definition of y and z.

Hyphal growth inhibition (GI) = 100 x [(y – z)/ y] where y = Mycelial growth of the fungal pathogen alone (control), z = Mycelial growth of fungi pathogens in PDA incorporated with culture filtrate.

Extracellular enzymes activities

Phosphate solubilization assay

The selected *Trichoderma* isolates were further tested for their ability to solubilize inorganic phosphate as described by Pikovskaya[31]. Briefly, autoclaved (121°C, 15 min) Pikovskaya's agar medium made up of 2.5 g Calcium Phosphate ($CaHPO_4$), 13 g glucose 0.5 g $(NH_4)_2SO_4$, 0.2 g NaCl, 0.1g $MgSO_4$-$7H_2O$, 0.5 g yeast extract, 0.2 g KCl, 0.0002 g $MnSO_4$, 0.0002 g $FeSO_4$-$7H_2O$, 10g Dextrose and 15g Agar per liter (pH=7.2) were poured in Petri dishes (90 mm). Mycelial plugs of each *Trichoderma* isolate (7 mm Ø) were cultured on the center of the plates and incubated at room temperature for 3 days. The solubilization of Phosphate was checked in the form of a clear colour halo around the colony.

Proteolytic activity

As mean to justify the mycoparasitic potential exhibited by the bioagents, the isolates were screened for their ability of produce extracelular proteases. For that, casein hydrolysis agar medium, containing 0.5 g KCl,

0.1 g $CaCl_2.2H_2O$, 0.2g $MgSO_4.7H_2O$, 1 g KH_2PO_4, 25 ml with 15% powdered skim milk, 10 g glucose and 12 g agar per Liter distilled water (pH= 5.4) was prepared as described by the manufacturer (APHA, Washington D.C.) and sterilized (121°C, 15 min) [32]. Mycelial plug (7 mm Ø) of each isolate, taken from the edge of growth on PDA plate were plated on fresh SKIM Milk agar medium and incubated for 3 days (28 ± 2°C). The proteases activity was positive upon the observation of a clearance zone around the colony.

Enzymatic scoring

The isolates were tested in triplicate and the overall experiment was repeated twice. The Enzymatic Activity Index (EAI) were calculated[33]

EA= [a + b] / a

Where : a = colony diameter, b : halo diameter.

Seed biopriming assays

Effects of bioagents cells free culture filtrates common bean seed germination

The culture filtrates from *Trichoderma* spp. (same as Section 2.3) were used to study their effects on the germination of two favourite commercial common bean varieties: GLP 190 (Mac-mac) and PH 201 (Meringue). Seeds with no cracks or other visible deformations were selected and surface sterilized for 10 minutes with 1 % sodium hypochlorite solution. Seeds were rinsed three times with sterilized distilled water and air dried. Dried bean seeds were deeped for 2 hours in culture filtrates from each *Trichoderma* isolate. For the untreated control, they were deeped in distilled water. Seeds were then air dried in the hood and aligned in Petri plates provided with two layers of Whatman filter paper N°1 soaked in sterile distilled water. The Petri dishes were then sealed with parafilm and incubated on laboratory bench at 25±2°C and 80% relative humidity[34]. Seeds were considered germinated upon radicle emergence. Germinated seeds were counted daily till total germination and the Mean Emergence Time (MET) was calculatedaccording to the equation from Ellis and Roberts[35]

MET=∑nidi/ n

Where: n is the total number of emerged seedlings during the emergence test; ni is the number of emerged seedlings on day d and i is the number of days during the emergence period. The germination pecentage was also recorded as the number of germinated seeds at the last counting day over the total number of seeds in each treatment [34].

Effects of bioagents conidia on common bean seed germination

In order to evaluate the effect of *Trichoderma* directly on seed germination, the surface disinfected seeds were soaked in the *Trichoderma* conidia suspension. For preparation of spore suspension, 5 mm diameter mycelia disc of 7 days-old culture obtained from the margin of each *Trichoderma* isolate was centrally placed on the surface of 100 ml PDA in a 250 ml conical flask and incubated at 25±1°C for 7 days. After the incubation period, 30 ml of sterile distilled water was added to each conical flask and shaken on a rotary shaker set at 80 rpm for 30 min. The concentration of spores of *Trichoderma* spp in dH_2O were counted using a haemocytometer and their concentration adjusted to 10^7 per ml. Seeds were dipped in in the spore suspension and placed on a sterile blotting paper in Petri dishes and incubated at 25±2°C in laboratory conditions after sealing with parafilm. Percentage of seed germination was evaluated after 5 days. The MET was calculated as described by Ellis & Roberts[35] and AOSA[36]

Greenhouse Experiments.

Phytostimulation assay

Effects of the *Trichoderma* isolates on bean seedlings growth was tested under greenhouse conditions at Antimicrobial & Biocontrol Agents Unit (AmBcAU), Laboratory for Phytobiochemistry and Medicinal Plants Studies, according to the modified method proposed by Mukhtar *et al*[37]. The soil used was collected from the A horizon of common bean farms around Yaoundé and was classified as Rhodic kanduidlut according to the U.S. soil

taxonomy. The soil sample was air-dried, passed through a 4-mm sieve before mixing with river sand (3:1 w/w) and autoclaved two times (121 ^{o}C; 1 h). The thus prepared substrate was then filled in 5 liters pot (12.5 x 17 cm). One hundred surface sterilized bean seeds were coated with suspensions of each *Trichoderma* isolate (10^{7}conidia/ml). Untreated control were dipped in distilled water. Dry *Trichoderma* coated seeds were sown in four replications (10 seeds per). The experiment was arranged in a completely randomized bloc design and was repeated twice. The seedling emergence percentages was taken after one week and the root and shoot lengths measured 28 days after sawing.

The vigour index was calculated according to the following formula by Asaduzzaman & Alam[38]:

Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] x percentage of seed germination.

***In vivo* biocontrol assay**

For the greenhouse biological control hability, *F. solani* was selected as it was the most virulent upon the pathogenicity test. Briefly, sterilized soil (same as above) was contaminated with *F. solani* conidia with a ratio of 3000 conidia/g of soil[9] and filled in pots (5 kg/pot). At sowing, surface sterilized bean seeds soaked in the BCA suspension (2 x10^{5} cells/mL) for 24 h were sown in each pot (10 seeds/pot). Pots contaminated by the pathogen only and non-treated ones (without fungal) were considered as controls. The pots were arranged in a greenhouse (28 ± 2^{o}C) in a randomized block designed in three replicates, cultivated and watered as needed. The treatments were as follows: *T. polysporum* T1+ *F. solani*; *T. atroviridae* T2 + *F. solani*; *T. sp.* T3 + *F. solani*; *T. harzianum* T8 + *F. solani*; *F. solani* alone and control (sterile soil alone). Four weeks after sowing, growth parameters (Emergence percentage, root and shoot length, root and shoot dry weight and vigor index) were recorded. Disease incidence (DI) was obtained by uprooting all the standing plants and counting the number of plants exhibiting root rot symptoms. This number was expressed as a percentage of the number of plants assessed. root rot severity (DS) was assessed by observing the roots and hypocotyls and scores given, based on a 1 to 9 disease scale developed at CIAT[9] as: 1= no visible symptoms; 3= light discoloration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions; 5 = approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system; 7 = approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting, and reduction of root system; 9 = approximately 75% or more of the hypocotyl and root tissues affected, with advanced stages of rotting combined with severe reduction in the root system. The DS percentage was then determined[39].

$$DS = \frac{\sum (ab) \times 100}{AK}$$

Where;

a = number of diseased plants having the same degree of infection, *b* = degree of infection, A = total number of examined plants and K = highest degree of infection (1-9 scale).

Statistical analyses

The data collected were analyzed using Sigmaplot statistical software (version 11.0). All the tested parameters were submitted to one way ANOVA, and differences between means were tested using the Tukey's multiple comparison based tests at 1% level of significance for the PBCI and 5% for any other experiment. The Pearson correlation was used to study the relationships between the tested parameters.

RESULTS

***In vitro* antagonism screening**

The PBCI and the percent reduction in growth of *F. oxysporum*, *F. solani* and *M. phaseolina* in response to antagonistic agents are represented in Tables 2, 3 and 4 respectively. The presented data showed that the growth of

pathogenic fungi was significantly reduced by the inhibitory action produced by all antagonistic agents tested ($p < 0.05$). On the other hand the mycelial growth inhibition of pathogenic fungi affected by the antagonistic fungi varied depending on antagonist-pathogen interaction.

Concerning *F. oxysporum*, the growth inhibition ranged between 57.6 to 72.1%. The maximum growth inhibition was recorded by isolate *Trichoderma* sp. T8 (72.1%) followed by *Trichoderma* sp. T3 (70.1%) and *Trichoderma* sp. T4 (69.2 %) respectively. Whereas the *Trichoderma* sp T10, T11 and T12 isolates were the least effective biocontrol agents (57.6%). The best biocontrol agent according to the Pakdamann index (PBCI = 0.053 cm^{-1}) was attributed to the *Trichoderma* sp. T8 isolate folowed by *Trichoderma* sp. T4 (PBCI= 0.050 cm^{-1}) (Table 2).

With *F. solani*, the inhibitory precentages ranged from 66.6 to 77.5% the most potent bioagent registered were *Trichoderma* sp. T1 and *Trichoderma* sp. T2 (77.5%) isolates followed by *Trichoderma* sp. T9 (75%), *Trichoderma* sp. T4 and T5 (74.5%). *Trichoderma* sp.T6 (66.6%) was the least active isolate (Table 3). By integrating the parameters C, Z, M, the *Trichoderma* sp. T1 (PBCI = 0.105 cm^{-1}) and *Trichoderma* sp. T2 and T4 isolates (PBCI = 0.086 cm^{-1}) were found to the best biocontrol agents on *F. solani*.

As with *F. oxysporum*, the best mycoparasitic fungus as concerns percentage inhibition against *M. phaseolina* was exhibited by the *Trichoderma* sp. T8 isolate (88.2%) upon the last data record, followed by *Trichoderma* sp. T1, T2 and T7 (85, 85, and 85.2% respectively). The Pakdaman index indicated that the *Trichoderma* sp. T8 (PBCI= 0.0750 cm^{-1}) and *Trichoderma* sp. T2 (0.0635 cm^{-1}) isolates showed prominent biocontrol ability on *F. oxysporum*, as compared to other *Trichoderma* species and the negative control. Considering the inhibition percentages, the parameter (C) and the PBCI (Tables 2, 3 and 4) on tested fungi, the overall biocontrol ability of the *Trichoderma* isolates was quantified and classified using the Tukey's based multiple comparison test ($\alpha = 0.001$). Indeed, the *Trichoderma* sp. T1, T2, T3 and T8 isolates were selected for further investigations after the identification at the species level.

Table 2: Mean C, Z, M, P and PBCI values of *Trichoderma* spp. and *F. oxysporum* in dual culture

Isolates codes	Mean C	Mean Z	Mean M	Mean P	*MGI	PBCI (cm^{-1})
Trichoderma sp. T1	3	6	3	18	65.3	0.027a
Trichoderma sp. T2	3	4	3	21	59.6	0.035b
Trichoderma sp. T3	2	4	3	15.5	70.1	0.048^{e}
Trichoderma sp. T4	3	5	4	16	69.2	0.050f
Trichoderma sp. T5	3	5	4	18	65.3	0.044d
Trichoderma sp. T6	4	0	4	21	59.6	UD
Trichoderma sp. T7	3	0	5	20	61.5	UD
Trichoderma sp. T8	2	4	3	14.5	72.1	0.053g
Trichoderma sp. T9	3	5	4	19.5	62.5	0.041c
Trichoderma sp. T10	4	0	4	22	57.6	UD
Trichoderma sp. T11	3	0	4	22	57.6	UD
Trichoderma sp. T12	3	0	4	22	57.6	UD
Control	-	-	-	-	00.00	-

Mean value followed by different alphabet letter within a column differ significantly over one another at $P< 0.001$ lead by Tukey Multiple Range Test. UD: undetermined. *percent mycelia growth inhibition. C = days required for the two antagonist colonies to come into contact; Z = days required for the BCA to fully grow over the pathogen colony; M = days required for the BCA to fully grow over the plate; P = the radial growth distance (in cm) of pathogen colony between the point of inoculation and the marginal point of contact with the BCA growth zone; PBCI = Pakdaman's biological control index.

Table 3: Mean C, Z, M, P and PBCI values of *Trichoderma* spp and *F. solani* in dual culture

Isolates codes	Mean C	Mean Z	Mean M	Mean P	*MGI	PBCI (cm^{-1})
Trichoderma sp. T1	3	6	3	19	72.4	0.105^{e}
Trichoderma sp. T2	3	4	3	15.5	77.5	0.086d
Trichoderma sp. T3	2	4	3	15.5	77.5	0.086d
Trichoderma sp. T4	3	5	4	17.5	74.6	0.071a
Trichoderma sp. T5	3	5	4	17.5	74.6	0.071a
Trichoderma sp. T6	4	0	4	23	66.6	UD
Trichoderma sp. T7	3	0	5	19.5	71.73	UD
Trichoderma sp. T8	2	4	3	16	69	0.083c
Trichoderma sp. T9	3	5	4	17	75	0.073b
Trichoderma sp. T10	4	0	4	21	69.5	UD
Trichoderma sp. T11	3	0	4	21	69.5	UD
Trichoderma sp. T12	3	0	4	21	69.5	UD
Control	-	-	-	-	00.00	-

Mean value followed by different alphabet letter within a column differ significantly over one another at P<0.001 lead by Tukey Multiple Range Test. UD: undetermined. *percent mycelia growth inhibition. C = days required for the two antagonist colonies to come into contact; Z = days required for the BCA to fully grow over the pathogen colony; M = days required for the BCA to fully grow over the plate; P = the radial growth distance (in cm) of pathogen colony between the point of inoculation and the marginal point of contact with the BCA growth zone; PBCI = Pakdaman's biological control index.

Table 4: Mean C, Z, M, P and PBCI values of *Trichoderma* spp and *M. phaseolina* in dual culture

Isolates codes	Mean C	Mean Z	Mean M	Mean P	*MGI	PBCI (cm^{-1})
Trichoderma sp. T1	3	6	3	17	85	0.0294a
Trichoderma sp. T2	3	4	3	12	85	0.0635f
Trichoderma sp. T3	2	4	3	15.5	81.7	0.0483c
Trichoderma sp. T4	3	5	4	14	83.5	0.0571^{e}
Trichoderma sp. T5	3	5	4	17	80	0.0470b
Trichoderma sp. T6	4	0	4	17	80	UD
Trichoderma sp. T7	3	0	5	12.5	85.2	UD
Trichoderma sp. T8	2	4	3	10	88.2	0.0750g
Trichoderma sp. T9	3	5	4	15	82.3	0.0533d
Trichoderma sp. T10	4	0	4	15	82.3	UD
Trichoderma sp. T11	3	0	4	18	78.8	UD
Trichoderma sp. T12	3	0	4	21	75.2	UD
Control	-	-	-	-	00.00	-

Mean value followed by different alphabet within a column differ significantly over one another at P< 0.001 lead by Tukey Multiple Range Test. UD: undetermined. *percent mycelia growth inhibition. C = days required for the two antagonist colonies to come into contact; Z = days required for the BCA to fully grow over the pathogen colony; M = days required for the BCA to fully grow over the plate; P = the radial growth distance (in cm) of pathogen colony between the point of inoculation and the marginal point of contact with the BCA growth zone; PBCI = Pakdaman's biological control index.

In vitro inhibitory effect of volatile metabolites

The volatile inhibitors of all selected *Trichoderma* isolates inhibited the mycelial growth of *F. oxysporum*, *F. solani* and *M. phaseolina* in a significant manner ($P < 0.05$) as compared to control plate (pathogens alone). In addition, the response varied between *Trichoderma* spp (Table 5). In general, the inhibition percentages ranged between 0.0 and 64.1%. Analysis of variance indicated either no differences (*M. phaseolina*

and *F. oxysporum*) or a statistically significant increase (*F. solani*) ($\alpha = 0.05$) in inhibition percentages of test pathogens over time (2, 5 and 8 days); indicating that at lower concentrations, *M. phaseolina* and *F. oxysporum* got adapted to the inhibitor and continued to grow through out the period of observation compared to the control while a substantial inhibition of *F. solani* growth was observed as the incubation time increased. *T. polysporum* T1 produced the most inhibiting volatile metabolites towards *M. phaseolina* and *F. oxysporum* no matter the incubation period. The highest inhibition being recorded after days 2 and 8 respectively with *F. oxysporum* (60.3%) and *M. phaseolina* (64.1%) whils *T. harzianum* T8 volatile inhibitors showed the best inhibitory potential against *F. solani* (57.6%) upon 8 days incubation.

***In vitro* inhibitory effect of non-volatile metabolites**

As it is obvious from Table 6, all non-volatile inhibitors of *Trichoderma* isolates also had significant differences with their respective controls in reducing the colony growth of *M. phaseolina, F. oxysporum* and *F. solani*. The inhibition percentages ranged between 16.5 and 72.6 %. The peason's correlation revealed a significant increase of growth inhibition with the incubation time ($r = 0.796$, 0.937 and 0.980; $p<0.05$ for *F. oxysporum, F. solani* and *M. phaseolina* respectively). Culture filtrate from *T. harzianum* T8 exhibited maximum growth inhibition against all tested fungi no matter the incubation period. The highest inhibition rate registered upon 8 days incubation was observed against *F. oxysporum* (72.6 %), followed by *F. solani* (68.4 %). The most resistant fungus was *M. phaseolina* (59.1%). *T. polysporum* T1 and *T. sp.* T3 where the least active isolates exibiting 31.5% and 20.7 % inhibition respectively against *F. oxysporum* and *F. solani* and 16.5% inhibition against *M. phaseolina* (Figure1).

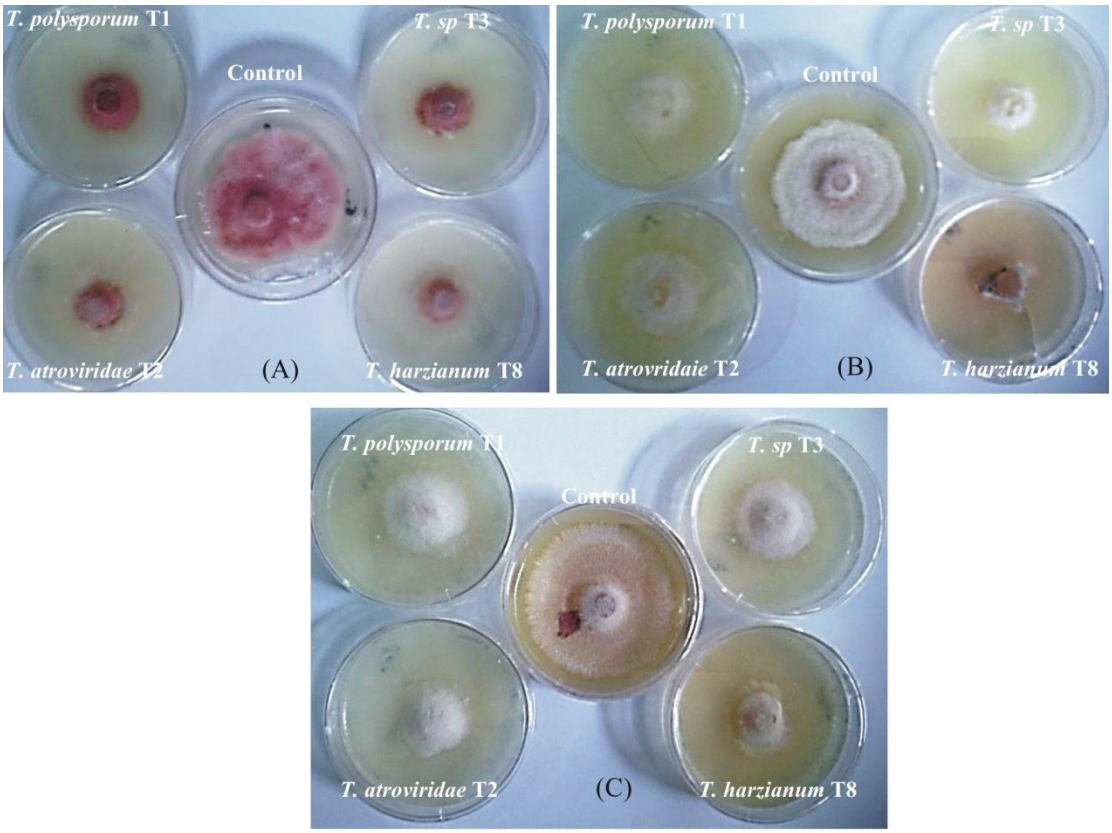

Fig. 1: Differential inhibitory effect of *Trichoderma spp.* culture filtrates on *F. oxysporum* (A), *F. solani* (B) and *M. phaseolina* (C), mycellia

Table 5: *In vitro* growth inhibition of *F. oxysporum, F. solani* and *M. phaseolina* by volatile compound from selected *Trichoderma* spp.

	Growth Inhibition (%)								
Treatments	***F. oxysporum***			***F. solani***			***M. phaseolina***		
	Day 2	**Day 5**	**Day 8**	**Day 2**	**Day 5**	**Day 8**	**Day 2**	**Day 5**	**Day8**
***T. polysporum*T1**	60.3 ± 2.4op	59.3 ± 6.3nop	37.6 ± 1.6ij	0.0 ± 0.0a	32.2 ±10.6ghi	37.6 ±1.6ij	52.6 ± 1.4klm	57.1 ± 1.0lmno	64.1 ± 0.8p
***T. atroviridae*T2**	24.1 ± 0.0de	26.4 ± 1.82efg	5.8 ± 0.0ab	0.0 ± 0.0a	17.1 ± 1.5c	52.9 ± 0.0klmn	24.2 ± 0.0de	30.8 ± 2.1fgh	39.4 ± 0.8j
***T.harzianum*T8**	18.9 ± 7.0cd	27.2 ± 6.2efgh	22.3 ± 6.6cde	4.5± 6.4ab	26.8± 0.0efg	57.6± 0.0lmnop	51.5 ± 0.0kl	58.6±1.0mnop	60.5 ± 0.8op
***T.* sp T3**	24.9 ± 3.6def	33.5 ± 0.9hij	32.3 ± 4.1ghi	9.0± 0.0b	23.6± 4.5cde	47.0± 3.3k	39.9 ± 1.4j	51.1 ± 1.0kl	62.3 ± 0.0op
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Mean	**32.05 ± 19**	**36.6 ± 15.4**	**24.5 ± 13.9**	**6.75 ± 3.1**	**25.2 ± 2.2**	**52.3 ± 7.4**	**42.05 ± 13.2**	**49.4 ± 12.8**	**56.5± 1.5**

Mean value followed by different alphabet letter (a,b,c,d,e,f,g,h,I,j,k,l,m,n,o,p,q) differ significantly over one another at P< 0.05 lead by Tukey Multiple Range Test

Table 6 : In vitro growth inhibition of *F. oxysporum, F. solani* and *M. phaseolina* by non-volatile compounds from selected *Trichoderma* spp

	Growth Inhibition (%)								
Treatment	***F. oxysporum***			***F. solani***			***M. phaseolina***		
	Day 2	**Day 5**	**Day 8**	**Day 2**	**Day 5**	**Day 8**	**Day 2**	**Day 5**	**Day8**
***T. polysporum*T1**	31.5±0.0cdefg	54.4±9.6lmno	54.1±10.9klmno	20.7±0.3bc	43±1.7ghijkl	48.94± 0.7ijklmn	31.5±7.4cdefg	42.8±8.0ghijkl	51,0±4.8jklmno
***T. atroviridae*T2**	34.2±3.7defgh	62.4±0.0opq	59.7±4.3nop	24.9±1.8bcde	48.1±5.3ijklmn	52.6± 10.4klmno	23.6±11.1bcd	38.5±2.0fghi	46.5±4.8ijkl
***T.harzianum*T8**	42.1±0.0ghijk	72.6 ± 3.2q	72.1±4.3q	36.8±7.4efghi	62.0±3.5opq	68.4± 2.9pq	39.4±3.7ghij	54.2±4.04klmno	59.1±0.0mnop
***T.* sp T3**	42.1±7.3ghijk	51.0±14.4jklmno	42.2±0.0ghijkl	26.3± 0.0bcdef	44.3±0.0hijkl	46.8± 0.7ijklm	16.5±12.3b	32.8± 6cdefgh	38.6±6.4ghi
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0±0.0a
Mean	**37.4± 5.4**	**60.1 ± 9.6**	**57 ± 12.4**	**27.1± 6.8**	**49.3 ± 8.7**	**54.1 ± 9.7**	**27.7 ± 9.8**	**42 ± 9.0A**	**48.8 ± 8.5**

Mean value followed by different alphabet letter (a,b,c,d,e,f,g,h,I,j,k,l,m,n,o,p,q) differ significantly over one another at P< 0.05 lead by Tukey Multiple Range Test

Extracelullar enzymes assay

As depicted in Table 7, all the tested bioagents could synthetise and release proteolytic and phosphate solubilization factors in specific solid media. The results being a fonction of involved isolates. For protease activity, the isolate *T.* sp T3 exhibited the highest activity (EAI=1.86) as compared to other isolates by showing the biggest clear zone on Skim Milk Agar. The results obtained for phosphate solubilization, pointed *T. polysporum* T1 (EAI= 1.50), T. sp T3 (EAI= 1.52) and *T.harzianum* T8 (EAI= 1.52) as main producers.

Table 7 : Proteolytic and Phosphate solubilization potential of *T. polysporum* T1, *T. atroviridae* T2, *T.* sp T3 and *T. harzianum* T8

Trichoderma spp.	Enzymatic Activity Index (EAI)		
	Proteolytic activity	Phosphate solubilization	Mean
T. polysporum T1	1.62 ±0.02[a]	1.5± 0,01[b]	**1.42**
T. atroviridae T2	1.59 ± 0.01[a]	1.23 ± 0.0[a]	**1.28**
T. sp T3	1.86 ± 0.01[b]	1.52 ± 0.02[b]	**1.5**
T.harzianum T8	1.66 ± 0.06[a]	1.52 ± 0.0[b]	**1.45**

Mean value (n=3) followed by different superscribed alphabet letter (a and b) within each column differ significantly over one another at P< 0.05 lead by Tukey Multiple Range Test.

Effect of *Trichoderma* spp. culture filtrates on common bean seed germination, and MET.

The results of the effect of culture filtrates from selected *Trichoderma* isolates on two popular common bean varieties are presented in Figure 2. Statistical analysis showed significant differences in seed germination percentage and MET of both varieties at α =0.05 levels as compared with non treated seeds (water control). The seed germination percentage ranged between 65 and 100%. *T. harzianum* T8, *T. sp.* T3 and *T. atroviridae* T2 culture filtrate exhibited higher enhancement of germination (100%) in bean seeds of the GLP190 variety upon the final count. *T. polysporum* T1 was less effective (95%) than others but significantly greater than the control (65%). The MET also was slowed down from 4.2 days (control) to 3.6, 4 and 4.05 days respectively after treament with *T. harzianum* T8, *T. sp.* T3 and *T. atroviridae* T2. A reverse trend was registered with filtrates from *T. polysporum* where an increase in MET was recorded (4.5 days) compared to control (Figure 3). With seeds of PH 201 variety, germination percentages ranged between 55 to 85%. *T. atroviridae* T2 and *T. polysporum* T1 culture filtrate exhibited highest enhancement of the germination percentage (85%) followed by *T. harzianum* T8 (65%). A controversal trend was recorded with *T. sp.* T3, where a reduction (55%) of markedly (p>0.05) seed germination rate were observed in relation to the control (60%) (Figure 2). However, no significant difference in MET was recorded after treating seeds with the culture filtrate from the overall *Trichoderma* spp.

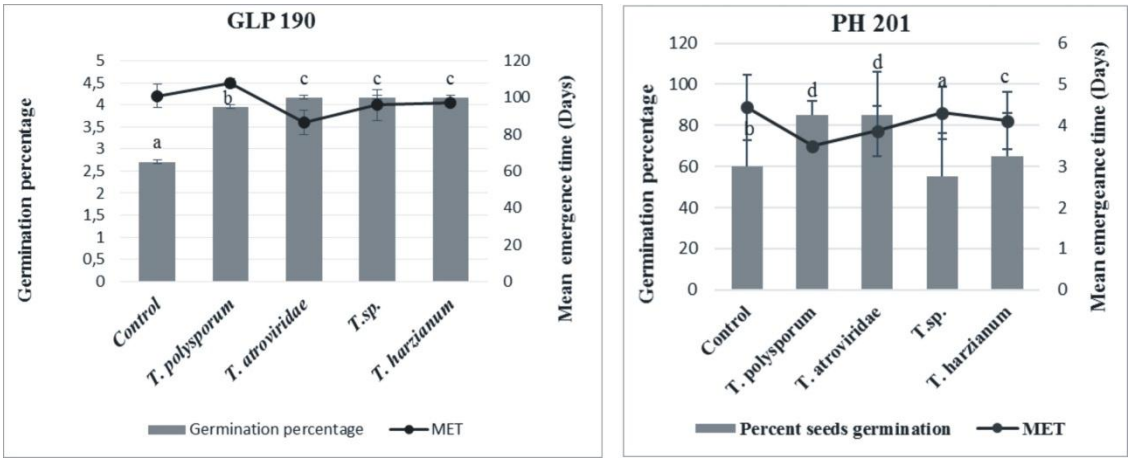

Fig. 2: Effect of *Trichoderma* spp. Culture filtrates on the germination and MET of two common bean varieties (GLP 190 and PH 201). Bar marked by the same alphabet letter are not significantly (P<0.05)

Fig. 3: Differential effect of bioagents culture filtrate on common bean seed germination

Effect of bean seed coating with *Trichoderma* spp conidia on percentage germination and MET

The result shows that both seed germination rate and speed varied in fonction of *Trichoderma* spp. and seed variety. Upon coating seeds of GLP 190 variety with *Trichoderma* spp conidia, the seed germination and germination speed percentage they did not vary significantly (P>0.05) in laboratory conditions. Indicating no side effects on seeds (Figure 4). However, enhancement of emergence percentages was registered under screenhouse conditions with *T. hazianum* T8 (94.16%) and *T. sp* T3 (74.16%) compared to untreated control (66.66 %). As for the blotted paper assay *T. atroviridae* T2 exibited no effect on been seeds (GLP 190) in the pot experiment (P > 0.05). On the other hand, *T.polysporum* reduced the emergence percentages of non-treated seeds. Elsewhere, seeds of the PH 201 variety coated with *Trichoderma spp*. registered an increase of the germinaton percentage which was high when treated with *T. hazianum* T8 and *T. atroviridae* T2 (100%) upon the final count. The least responses however higher than the water control (85%) were observed with *T.polysporum* (95%) and *T. sp*. T3 (95%). The germination speed was identitical with bean seeds treated with *T. hazianum* T8 (2.3 days) and *T. sp*. T3 (2.1 days) compared to control (2.01 days). In contrast, *T. atroviridae* T2 (2.7 days) and *T.polysporum* T1 (2.7 days) reduced the germination velocity of bean seeds of the PH 201 variety (Figure 4b).

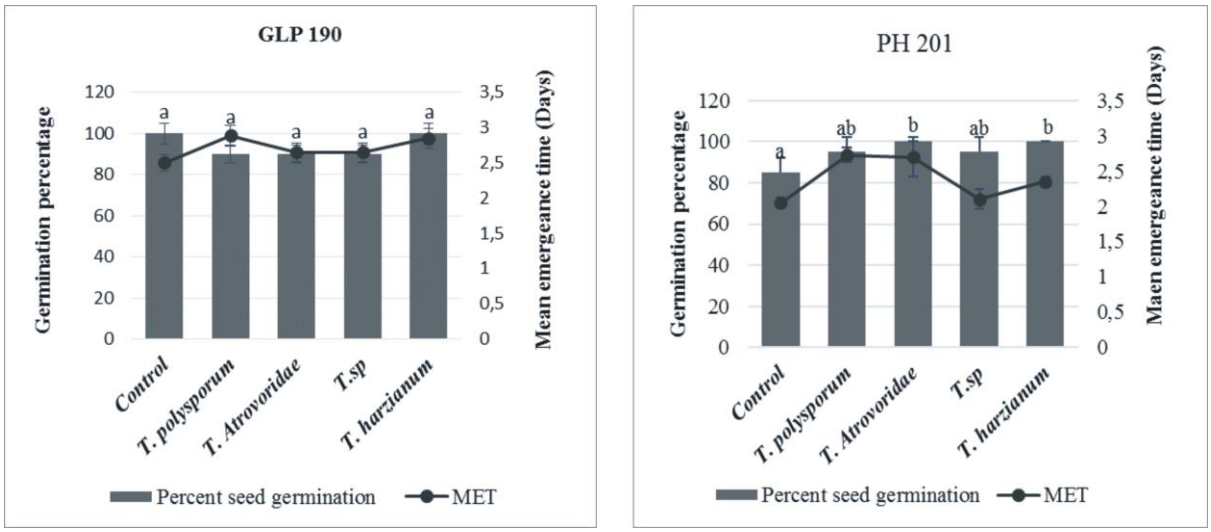

Fig.4: Effect of *Trichoderma* conidia on the germination and MET of two common bean varieties (GLP 190 and PH 201). Bar marked by the same alphabet letter are not significantly (P<0.05)

Phytostimulatory effect of *Trichoderma* isolates

The results of the greenhouse experiments showed that by treating bean seeds with *Trichoderma* spp, the shoot and root length were greater than those of untreated control.

The plant height of *Trichoderma* spp treated bean plants ranged from 53.4 to 71.8 cm whilst the mean length for the control was recorded at 46.6 cm. *T. hazianum* T8 had the highest ability to stimulate seedling shoot elongation (71.8 cm) as compared to other strains (Figure 5).The root length ranged between 23 and 32.74 cm. The highest value (32.74 cm) was registrered with *T. sp.* T3 which was the most promising isolate in root length stimultion while the control (23cm) was statistically equal to the seedling treated with *T. atroviridae* T2 (23.7cm).The results related to the vigour index showed similar changes as in germination percentages. Seed treatment with culture filtrates of *Trichoderma* strains increased vigour index compared to control. The most vigorous seedligs were recorded after coating seed with *T. harzianum* T8 (8128.17). The lowest vigour index was recorded in control (4639.53).

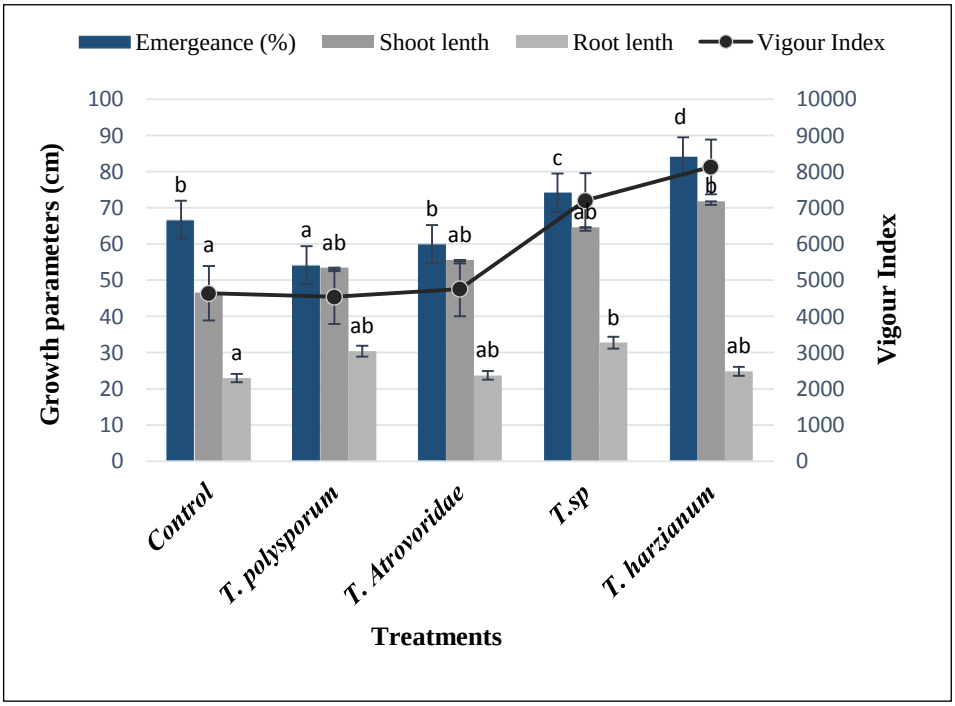

Fig. 5: Effect of seed treatment with four *Trichoderma* isolates on the shoot and root length of common bean seeds in pot conditions. Vertical bars show standard error of means of five replicates. In each parameter, bar marked by the same alphabet letter are not significantly (P<0.05) different (Turkey grouping analysis)

Effect of single and dual inoculation of *F. solani* and selected *Trichoderma* spp. on the agronomical parameters of *Phaseolus vulgaris* L.

The results presented in Table 8 indicate the beneficial impact of treatment of bean seeds with *Trichoderma* spp. compared to the deleterious effects of infection with *F. solani*. However, the negative impact of *F. solani* on the pre-emergence damping-off and the growth of *P. vulgaris* (P > 0.05) was corrected by treatment with the biocontrol agents. Thus, bean plants treated with all the antagonists grew faster with optimized agronomical parameters (enhanced root and shoot length and dry weights) and higher vigor index compared to seedlings inoculated with *F. solani* alone. This is an indication that *Trichoderma* treatments boosted plant metabolism leading to more tissue formation. Of note, the vigor index ranged between 3638.5 and 5840. Maximum vigor indexes

were observed in plants treated with *T. harzianum* T8 (5840) followed by *T. sp.* T3 (5740) with respective magnifications of about 1.6 and 1.57 fold on average compared to results obtained with *F. solani* treated plants.

Table 8. Effects of bioagents antagonist fungi on the growth of *P. vulgaris* plants inoculated with *F. solani* f. sp. *Phaseoli* under pot conditions

Treatments	Emergence (%)	Root lenth (cm)	shoot lenth (cm)	root dry weight (g)	shoot dry weight (g)	vigor index
Control	85 a	19.7 ± 1.7b	38.4 ± 3.5c	0.26 ± 0.02b	1.3±1.3d	5810
F. solani	95 bc	12.5 ±1.0a	25.8 ±1.6a	0.16 ± 0.02a	0.5 ± 0.5a	3638.5
T. polysporum T1+ *F. solani*	90 ab	20.4 ± 3.0b	31.9 ± 3.3b	0.26 ± 0.03b	0.6 ± 0.07ab	4445.5
T. atroviridae T2 + *F. solani*	95 bc	21.8 ±1.9b	32.7 ± 1.2b	0.29 ± 0.08b	0.8±0.08b	5177.5
T. sp. T3 + F. solani	100 c	24.7 ± 2.6c	32.7 ± 2.7b	0.36 ± 0.03c	0.9±0.16c	5740
T. harzianum T8 + *F. solani*	100 c	25.2 ± 3.5c	33.2 ±1.5b	0.31 ± 0.04bc	1.1±0.2d	5840

Mean value followed by different alphabet letter (a,b,c,d) in the column differ significantly over one another at P < 0.05 lead by Tukey Multiple Range Test

In vivo effeciency of the bioagents on disease incidence and severity

Bean plants inoculated with *F. solani* f sp. *phaseoli* only, clearly showed typical symtoms of fusarium root rot, characterized by substantial reductions in plant growth and higher leaves chlorosis. Seeds dressing with all the biocontrol agents significantly lowered ($p < 0.05$) the extent of both the disease severity and incidence induced by the pathogen on emerging plants (Table 9). The protection rate being significantly different ($P < 0.05$) among *Trichoderma* spp. In this regards, seeds coated with *Trichoderma*. sp. T3 was the best as it showed up to 50% and 46% reduction of DI and DS respectively. While, *T. harzianum* T8 treated plants exhibited highest disease indexes (0 % and 16.6 % reductions of DI and DS respectively) as compared with overall biocontrol agent.

Table 9. Effects of bioagents antagonist fungi on common bean root rot disease incidence and severity under greenhouse condition 28 DAS

Treatments	Disease incidence (%)	Reduction (%)	Disease severity (%)	Reduction (%)
Control	0.0a	-	0a	-
F. solani	100.0^{e}	-	90.0f	-
T. polysporum T1+ F. solani	66.6c	33.3	60.0c	30.0
T. atroviridae T2 + *F. solani*	77.7d	22.2	77.7e	12.2
T. sp. T3 + F. solani	50.0b	50.0	44.0b	46.0
T. harzianum T8 + *F. solani*	100.0^{e}	0.0	73.3d	16.6

Mean value followed by different alphabet letter in the column differ significantly over one another at P< 0.05 lead by Tukey Multiple Range Test

DISCUSSION

The harmful effects of chemical fongicides on both environment and human, in concomittancy with its controversial efficiency on rhizospheric diseases has made biological control a genuine alternative in controlling such disease. In addition to their wide spectrum towards plant pathogenic fungi[15, 16, 13]. The enhanced growth response of several plants species following application of *Trichoderma* spp. further favours their usage as biocontrol agents since the improvement of plant nutrition and vigour have been pointed out as an indirect mechanism involved in root disease suppression mediated by antagonistic microorganisms.

The major objective of this work was to investigate whether bean plant growth promotion and protection could be achieved through application of endophytic *Trichoderma* species isolated from common bean root. From the biological control perspective, it was demonstrated that overall isolates showed clear antagonistic effect by significantly inhibiting the growth of *F. solani*, *F. oxysporum* and *M. phaseolina*, the causative agents of common bean root rot/wilt disease. Nevertheless, the velocity and ability to overgrow and parasitize the mycelia of tested pathogems varied among *Trichoderma* spp. these observations strengthened the use of time parameter (C, M, Z; PBCI) as a selecting criteria in this study[26] in which *T. polysporum* T1, *T. atroviridae* T2, *T. sp* T3 and *T. harzianum* T8 showed the overall best potential as antagonist of tested soil pathogenic inhabitants. Such inhibitory effects has been thought to arise through the ability of *Trichoderma* species to grow much faster than the pathogenic fungus thus competing efficiently for space and nutrients and forming coiled structures around the hyphae of the pathogenic fungi. Starvation, a competition for nutrients has been the most common cause of death of phytopathogens[40]. Moreover, coiling is characteristic of the interaction between mycoparasitic and phytopathogenic fungi, leading to penetration of the cell wall of pathogen followed by the breakdown of chitin by production of chitinase and glucanase enzymes at the site of endophyte penetration[41,42]. The biosynthesis of these proteolytic enzymes by the selected bioagents could clearly been observed in this study throught the halo zones drawn on Skim Milk Agar medium. Further, the released antibiotics permeate the affected hyphae and inhibit resynthesis of the phytopathogenic cell wall. As refered to antibiotics, a wide range including trichodermin, trichodermol, herzianolide, ethylene and formic aldehyde have been reported[43]. Hence, the degree of effectiveness could be the result of the nature, quality, and quantity of antibiotics/inhibitory substances secreted by the antagonists[44]. Understanding the role of produced substances in mechanism (s) involved in the antagonistic effect of *Trichoderma* spp on plant pathogens is therefore an important tool in designing effective and safe biocontrol strategies[45]. Accordingly, the selected isolates produced volatile and non volatile compound which varied in their inhibitory effect against test fungi. *T. harzianum* T8 being the most effective. Theses results clearly or in part justified the inhibitory effects of the bioagents upon dual cultures assays and corroborate earlier findings that associate the production of nonvolatile and volatile metabolites with the ability of *Trichoderma* to control plant pathogens[46, 47].

Our results also provides evidence that cell free culture filtrates or conidia from selected biocontrol agents exhibited either inhibitory (*T. sp.* T3, and *T. polysporum* T1) or stimulating effects (*T. harzianum* T8 and *T. atroviridae* T2) on bean seeds (GLP 190 and PH 201 varieties) under screenhouse and blotted paper assay. These results demonstrated the pathogenicity of this genus to germinating seeds and seedlings although *Trichoderma* spp. have rarely been regarded as parasites. Celar & Valic[48] supported this observation by showing a significant inhibition of onion seed, chicory and lettuce germination by *T. viride* culture filtrate, and *T. koningii* respectively in the blotted paper assay. Globally, *T. harzianum* T8 gave early germination as well as high germination percentage *in vitro*, the emergeance percentage, root and shoot length as well as seedling vigour in greenhouse condition more than other isolates ($P<0.05$). In fact, induction of plant growth and vigour mediated by *Trichoderma* spp. is not well understood. Nevertheless, Doni *et al*[18] related the growth promotion to their ability to trigger the exudation of plant growth stimulating factors and phytohormones like indole acetic acid (IAA) and their analogs, siderophores and phosphate-solubilizing enzymes. These often leads to solubilization and increase in some nutrients resulting in stranger nutrients uptake

by plant, as well as indirectly controlling the minor root infested pathogens.

Meanwhile, results indicated that all antagonist species significantly reduced ($p < 0.05$) the Disease Severity and Incidence as well as increased in all growth parameters in pot conditions, highlighting the potential of *Trichoderma* spp. to enhance germination and the frequency of healthy plants and vigor index in infested soils. However, given that the growth parameters of healthy plant treated with bioagents were more favorable than those of infected plants also treated with the same bioagents, the growth promotion should not be related to the biological control ability. But, it is likely that *Trichoderma* spp create more favorable growing conditions which lead to a better plant growth. The results were in agreement with the findings of other researchers who suggested that in addition to their biocontrol ability, some *Trichoderma* species are able to promote plant growth[49, 47]. It is noteworthy that *Trichoderma* hyphae releas elicitors which contribute in signal transmition within the plant such as salicylic acid (SA), jasmonic acid (JA) and reactive oxygen species (ROS) [50]. These elicitors being taught to be involved in triggering expressions of defense metabolites within the plant[51]. In this way, plant immunity against pathogens.

CONCLUSION

Seed priming has become fashionable among agricultural practitioners in the effort to improve crop vigour under normal and adverse conditions, particularly in infected agricultural soil. Indeed, this study confirmed that endophytic *Trichoderma* spp. from *P. vulgaris* display antagonistic activity against *F. oxysporum*, *F. solani* and *M. phaseolina* found in wilted and roted bean roots through physical attack and or by releasing metabolites/compounds in their neighbouring environment. Even though it is true that under field conditions there are other biotic or abiotic factors that may influence the expression of the antagonism, the *in vitro* assays allow the selection of *Trichoderma* species suitable for biocontrol and plant growth promoter but further research is needed to validate the most prominent strains under systems that resemble natural conditions or under field conditions.

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