Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Biological control potential of *Trichoderma asperellum* against *Pseudocercospora griseola*, the etiological agent of angular leaf spot in common bean (*Phaseolus vulgaris* L.)

Gael Chatue Chatue[1,2]*, Clive Neba Akongnwi[3], Junior Frank Nkodo Mvongo[2], Marie Amperes Boat Bedine[4], Alain-Martial Sontsa-Donhoung[1], Pascal Touna Touna[1], Patrick Fogang Kenfack[1], Christopher Suh[5], Aime Didier Begoude[1], Fabrice Fekam Boyom[2]

[1] Crop Protection, Institute of Agricultural Research for Development (IRAD), Yaoundé, Cameroon

[2] Department of Biochemistry, University of Yaoundé, Yaoundé, Cameroon

[3] Post-Harvest Unit, Ekona Agricultural Research Centre, Institute of Agricultural Research for Development (IRAD), Buea, Cameroon

[4] Department of Agriculture, University of Dschang, Dschang, Cameroon

[5] Annual Crop, International Institute for Tropical Agriculture (IITA), Kinshasa, Congo

DOI: 10.24425/jppr.2026.158978

Received: July 16, 2025
Accepted: September 23, 2025
Online publication: May 06, 2026

*Corresponding address:
chatuegael88@gmail.com

Responsible Editor:
Piotr Iwaniuk

Abstract

Angular leaf spot disease, which is caused by the fungus *Pseudocercospora griseola*, is among the most damaging diseases affecting common bean (*Phaseolus vulgaris* L.), impacting both yield and grain quality. Because of the environmental risks associated with fungicides and the variability in the virulence of *P. griseola* isolates, biological control emerges as a promising alternative for managing this disease. This study assessed the biological control potential of *Trichoderma asperellum* 659-7, PR11, and PR12 against *P. griseola*. Additionally, changes in some biochemical parameters were also investigated. The findings revealed that the three tested strains stopped the growth of *P. griseola* during the confrontation test, achieving 100% inhibition. Furthermore, the cell-free culture filtrates from each *T. asperellum* strain hindered the mycelial growth and spore germination of *P. griseola*, with the level of inhibition depending on both the concentration of culture filtrate and the specific strain of *T. asperellum*. The most significant reduction was noted with *T. asperellum* PR11, which decreased mycelial growth by 26.33% and spore germination by 27.14% at 25% (v/v). Moreover, treating infected bean leaves with *T. asperellum* PR11 led to a reduction in disease severity by 11.32 and 22.5% at 14 and 21 days after inoculation, respectively. An increase in chlorophyll content (287.087%), total phenols (43.116%), and flavonoids (72.010%) was also observed when infected leaves were treated with *T. asperellum* PR11. These overall results endorse the effectiveness of *Trichoderma asperellum* PR11 as a biological control agent for managing bean angular leaf spot, offering an alternative and environmentally friendly strategy.

Keywords: *Pseudocercospora griseola, Phaseolus vulgaris*, *Trichoderma asperellum*

Introduction

The common bean (*Phaseolus vulgaris* L.) is among the most significant cultivated legumes in the world (Broughton *et al*. 2003), with an estimated annual production of 27.5 million tons in 2022 (FAO 2023). This important legume consumed worldwide serves as an essential source of protein, minerals, antioxidants, and bioactive compounds (Karavidas *et al*. 2022). The Common Bean Observatory (CBO) reported an

estimated annual production of 28.5 million tons of bean in 2023 (CBO 2025), 43.9% of which come from Asia, followed by North and South America (32.2%), and Africa (21.3%) (FAO 2023). According to the Global Dry Beans Production by Country 2025, Myanmar was the first producer of this legume in 2023, followed by India, Brazil, Tanzania, and China. In Cameroon, bean production in 2023 reached 390,098 tons, harvested from an area of 303,828 hectares, resulting in an average yield of 1.28 tons per hectare (CBO 2025). This yield is significantly below the plant's potential, which is approximately 2 tons per hectare, with some varieties capable of producing up to 3.2 tons per hectare (Kebede 2021; Nchanji *et al.* 2023).

The leading causes of this poor production are low soil fertility, dryness (Papathanasiou *et al.* 2022), insect attacks (Adomako *et al.* 2022), and diseases (Eke *et al.* 2019). Within these diseases, angular leaf spot (ALS) which is caused by the phytopathogenic fungi *Pseudocercospora griseola* (Sacc.) Crous & U. Braun is considered the most destructive disease affecting bean in Latin America as well as in Africa (Sartorato 2004; Crous *et al.* 2006). This disease occurs in about 80 countries in the main bean-growing regions worldwide (Ddamulira *et al.* 2014; Nay *et al.* 2019; Bi *et al.* 2023), and it is characterized by angular dark to gray spots on the stem, leaves, and pods of the bean plant (Taboada *et al.* 2022). The disease's incidence has gone up recently, resulting in significant economic losses due to monocultures and the limited genetic diversity of commercial bean varieties (Taboada *et al.* 2022). Otherwise, when environmental conditions are favorable for the pathogen and a susceptible variety is cultivated, up to 80% of the total crop production can be lost because of the disease (Nay *et al.* 2019; Wani *et al.* 2022).

Numerous control methods, including the use of fungicides, disease-free seeds, crop rotation, host plant resistance, and cultivar mixtures, have been utilized to control ALS (Ddamulira *et al.* 2014; Ddamulira 2019). However, the emergence of different pathogen races that could overcome host resistance, coupled with the lack of suitable resistance sources often compromises genetic resistance, although it is effective and affordable (Abadio *et al.* 2012; Ddamulira *et al.* 2014; Palacıoğlu *et al.* 2021). As a result, tremendous efforts have been made worldwide to find new alternatives and reduce the negative impacts of chemicals. One promising method which has minimal environmental impact is biological control (O'Brien 2017). This approach utilizes the antagonistic mechanisms of biocontrol agents such as antibiosis, mycoparasitism and competition to disrupt the pathogen's life cycle, reduce host tissue colonization, and thereby interfere with the pathogen's ability to survive (Punja and Utkhede 2003; Busby *et al.* 2016). Among biocontrol agents, endophytic fungi such as *Trichoderma* species, known to be the most important filamentous fungi used for biological control of phytopathogens (Mohiddin *et al.* 2010; Poveda *et al.* 2020), have drawn interest (O'Brien 2017; Hyde *et al.* 2019). The biocontrol strategies of *Trichoderma* are based on stimulating several mechanisms, either directly through mycoparasitism, the secretion of active molecules, antibiosis, or indirectly through competition for nutrients and space, which promotes plant growth and enhance plant defense systems (Benítez *et al.* 2004; Ferreira and Musumeci 2021). They are considered promising candidates for biological control of plant diseases due to their diversity in producing a wide range of metabolites and their ability to inhibit the activity of up to 80% of some economically important plant pathogens (Phoka *et al.* 2020; Sánchez-Montesinos *et al.* 2020). Several investigations have demonstrated the antagonistic potential of *Trichoderma* species against pathogens such as *Fusairum solani* (Toghueo *et al.* 2016; Eke *et al.* 2020), *Uromyces appendiculatus* (Abeysinghe 2009) and *Rhizoctonia solani* (Mayo-Prieto *et al.* 2020), which cause root rot, rust and web blight in common bean, respectively. However, there are few studies demonstrating the biocontrol potential of *Trichoderma* against ALS of bean, and no study has investigated the antagonistic potential of the strains 659-7, PR11, and PR12 of *Trichoderma asperellum* against *P. griseola*.

Keeping all this in view, this study was carried out to: (1) evaluate the *in vitro* interactions between *T. asperellum* strains and *P. griseola*, (2) assess the *in vivo* efficacy of the most promising strain against ALS disease, and (3) evaluate the impact of both single and dual inoculation of bean leaves with *P. griseola* and *T. asperellum* on the chlorophyll content, total phenols and flavonoids content in bean leaves.

Materials and Methods

Source of the fungal pathogen and biological control agents

The fungal pathogen *Pseudocercospora griseola* used in this study was provided by the Antimicrobial & Biocontrol Agents Unit of the Laboratory for Phytobiochemistry and Medicinal Plants Studies of the University of Yaoundé 1. It was previously isolated from infected leaves showing the symptoms of ALS disease in a field in the Ndé division of the western region of Cameroon (5°10'28.8 "N and 10°33'58.9 "E). This isolated pathogen was the most aggressive and its identification was confirmed using molecular analysis (Gael *et al.* 2025).

Three strains of *Trichoderma asperellum*, namely *T. asperellum* 659-7, PR11 and PR12 from the core

collection of the Regional Laboratory of Biological Control and Applied Microbiology of IRAD were used as biological control agents (BCA). These strains were isolated from the top 8 cm of agricultural soils in Cameroon. Genotyping of these strains based on sequencing of translation elongation factor 1-alpha (tef1) was reported, and the sequences were deposited in GenBank. Their Genbank numbers are EF186002, EF185999 and EF186001 for *T. asperellum* PR11, PR12 and 659-7, respectively (Begoude *et al*. 2007). The antagonistic potential of these strains against various fungal pathogens, including *Phytophthora megakarya* and *Pythium myriotylum*, causative agents of black pod disease of cocoa and cocoyam root rot disease, respectively, has already been demonstrated (Tondje *et al*. 2007; Mbarga *et al*. 2012; Tchameni *et al*. 2017).

In vitro Trichoderma asperellum – *Pseudocercospora griseola* interactions. Direct confrontation test between *Trichoderma asperellum* strains and *Pseudocercospora griseola*

The *in vitro* antagonistic activity of *T. asperellum* 659-7, PR11 and PR12, against *P. griseola* was evaluated using the direct confrontation test on potato dextrose agar (PDA, Himedia) medium, with the Comporta (1985) procedure. Every plate was incubated for 45 days at 25±2°C. Ater measuring the pathogen's radial growth, the percentages of inhibition of mycelial growth were calculated according to formula (1) given by Mokhtar and Aid (2013).

$$I\,[\%] = (1 - \frac{T}{C}) \times 100, \quad [1]$$

where: *I* [%] – percentage of inhibition, C – radial growth of *P. griseola* in the control group, and T – radial growth of *P. griseola* cultured with the antagonist.

Effect of cell-free culture filtrates of *Trichoderma asperellum* strains on the mycelium growth of *Pseudocercospora griseola*

The effect of cell-free culture filtrates of the three *T. asperellum* tested on *P. griseola* mycelial growth was evaluated using the food poisoning method (Grover and Moore 1962). Cell-free culture filtrates of *Trichoderma asperellum* 659-7, PR11, and PR12 were prepared by inoculating sterile HIMEDIA® potato dextrose broth (100 ml) with a 5 mm diameter disc of young mycelial agar plugs of each actively growing *T. asperellum* strain at 150 rpm in a rotary shaker (WiseShake®) for 10 days at 25 ± 2°C. The cultures were then filtered through Whatman filter paper No. 1 to remove the mycelium, and the culture filtrate was sterilized by passing through 0.20 µm millipore microbiological filter membranes. Melted PDA medium (at 45 ± 5°C) was amended with 12.5% and 25% (v/v) of the cell-free culture filtrate of each *T. asperellum* strain and poured into Petri plates. After cooling and solidification, a 7 mm pathogen mycelial disc was centered on the plate for 60 days in the dark. The Petri dishes with sterilized PDA without cell-free culture filtrates served as the control, and three replicates were performed for each treatment. The pathogen's colony's diameter was determined, and inhibition of mycelial growth of *P. griseola* by cell-free culture filtrates of the biocontrol agents was evaluated according to the following formula (2):

$$MGI\,[\%] = \frac{(A-B)}{A} \times 100, \quad [2]$$

where: *MGI* – the mycelial growth inhibition, *A* – the radial growth of *P. griseola* in the control group, and *B* – to the growth of *P. griseola* in the treated group (Sreedevi *et al*. 2011).

Effect of cell-free culture filtrates of *Trichoderma asperellum* strains on the spore germination of *Pseudocercospora griseola*

To evaluate the impact of cell-free culture filtrates from *T. asperellum* 659-7, PR11 and PR12 on spore germination of *P. griseola*, the M38-A2 broth macrodilution assay described by Toghueo *et al*. (2016) was utilized. Glass tubes of 1.5 ml containing 500 µl and 625 µl of sterile potato dextrose broth (PDB, Himedia) mixed with 250 µl and 125 µl cell-free culture filtrates of *T. asperellum*, respectively. Then, a conidial suspension of *P. griseola* (250 µl) prepared at 2×10^4 spores/ml was added to each tube to complete the volume to 1 ml. Three replications were performed for each treatment, with the tubes containing PDB medium and *P. griseola* serving as the negative control. After 48 h of incubation in the dark at 25±2°C, the mixture was examined microscopically. Conidia with elongated germ tubes were considered germinated, and formula 3 below was used to calculate the percentage of inhibition of the germination of conidia (*ICG*%).

$$ICG\,[\%] = \frac{(G_c - G_t)}{G_c} \times 100, \quad [3]$$

where: *ICG* [%] – percentage of inhibition of conidia germination, G_c – germination in control tubes and G^t – germination in treated tubes.

In vivo evaluation of the selected *Trichoderma asperellum* PR11 strain for the biological control of angular leaf spot

Seed planting and inoculum preparation

Bean seeds of susceptible cultivar GLP 190 (Gael *et al.* 2025) were surface sterilized by soaking in 3% (v/v) sodium hypochlorite (NaOCl) solution for five minutes. Afterwards, they were rinsed three times with sterilized water before being sown in plastic pots (4 cm deep) filled with 5 kg of a sand-soil mixture (1 : 3 v/v) that had been sterilized at 121°C for 1 h in an autoclave. For each treatment, three pots were used, with four bean seeds sown in each pot. The pots received daily watering until the first trifoliate leaf was entirely developed.

An adjusted version of the procedure outlined by Pastor-Corrales *et al.* (1998) was used to produce the spore suspension of *P. griseola*. Sterile distilled water was added to 14-day-old cultures of *P. griseola* that were cultivated in PDA medium, and the spores were released by scraping the medium's surface with a flame-sterilized spatula. A pair of cheesecloth layers was employed to filter the resultant suspension, and a hemocytometer was used to determine its concentration. Sterile distilled water was then added to adjust the concentration to 2×10^4 conidia per ml.

Pathogen inoculation and treatment

Seventeen days after sowing, the first trifoliate leaves of each plant were sprayed on the upper and lower sides with the spore suspension of *P. griseola* (2×10^4 conidia/ml) till running off. The plants were then placed in a highly humid chamber (relative humidity > 90%) for three days to induce sporulation and subsequently transferred to a greenhouse (Castellamos *et al.* 2016).

Before treatment with the BCA, spore suspensions of *Trichoderma asperellum* PR11 strains were prepared using a seven-day-old culture of the strain grown on PDA, and the concentration of the suspension was adjusted to 10^8 conidia/ml using sterile distilled water. Ten days after infection with *P. griseola*, when symptoms began to appear, both the top and the bottom surfaces of the bean leaves were sprayed with *Trichoderma* spore suspension. The control groups included plants treated with sterilized distilled water (uninoculated control) and those infected with the pathogen only (untreated control). For each treatment, three pots (12 plants) were used, and the experiment was conducted in a completely randomized block design. Treatments were coded as follows: Control = plants treated with sterile distilled water; Pg = plants infected with *P. griseola* only; and Pg + PR11 = plants infected with *P. griseola* and treated with *Trichoderma asperellum* PR11.

Disease rating

At 14 and 21 days following infection with *P. griseola*, disease parameters were assessed. Disease incidence represented the total number of diseased plants as a percentage of all plants in each treatment (Nutter *et al.* 1991), and disease severity was assessed visually according to the 1–9 CIAT scale described by Van Schoonhoven and Pastor-Corrales (1987) and modified by Cajiao *et al.* (2023). Here, 1 = absence of visible symptom; 3 = presence of small lesions (less than 2 mm) that cover about 2% of the leaf area; 5 = presence of more typical medium-sized, angular lesions (≥ 4 mm) that make up about 5% of the leaf area; 7 = a lot of big, sporulation-filled lesions that take up almost 10% of the leaf area. The affected leaves turn chlorotic, and lesions grow on the underside of the leaves, forming gray patches with a lot of sporulation; and 9 = coalescent lesions that cover at least 25% of the leaf area and have high, gray-colored sporulation on the leaf undersides. Defoliation from chlorotic leaflets is strong and occurs early.

Effect of inoculation of bean leaves on some biochemical parameters

Total chlorophyll content

With minor adjustments, the method outlined by Arnon (1949) was used to quantify the amount of chlorophyll. For extraction, 10 ml of ice-cold 80% acetone (v/v) was used to motor-crush 0.5 g of the first trifoliate bean leaf of each treatment. Following a 5-minute centrifugation at 10,000 rpm, the mixture was collected, and the optical density (OD) of the supernatant for chlorophyll a and b was analyzed at wavelengths of 645 and 663 nm, respectively. The chlorophyll content for each instance was determined using formulas 4 through 6 below:

$$\text{Chlorophyll a [mg/g]} = \frac{[12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645)] \times \text{V} \times \text{W}}{1000}, \quad [4]$$

$$\text{Chlorophyll b [mg/g]} = \frac{[22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663)] \times \text{V} \times \text{W}}{1000}, \quad [5]$$

$$\text{Total chlorophyll [mg/g]} = \frac{[20.2\ (\text{OD } 645) - 8.02\ (\text{OD } 663)] \times \text{V} \times \text{W}}{1000}, \quad [6]$$

where: V – volume of the extract [ml], W – weight of the fresh leaf [g] and OD – optical density.

Extraction of total phenolic compounds and quantification

The method modified by Mujica *et al*. (2009) was used to extract total phenols.

The Folin-Ciocalteau method developed by Singleton and Rossi (1965) was used to quantify total phenolic compounds. The absorbance was measured at a wavelength of 765 nm, and the findings were reported as micrograms of gallic acid equivalent (GAE) per gram dry weight. Gallic acid was used as a standard.

Total soluble flavonoids

The method developed by Dewanto *et al*. (2002), which uses catechin as a standard, was used for flavonoids quantification. After thoroughly mixing the solution, the absorbance was determined at a wavelength of 510 nm. The µg catechin equivalents (CE)/g of dry weight was used to express the total flavonoid content (TFC).

Statistical analysis

All statistical analyses were performed with XLSTAT 2016 software. The raw data collected underwent analysis of variance (ANOVA), and Duncan's Multiple Comparison Test was applied at 5% significance level with 95% confidence limits to assess the differences between the means.

Results

Antagonistic activity of *Trichoderma asperellum* 659-7, PR11, and PR12 against *Pseudocercospora griseola*

The results of the direct confrontation test (Table 1) showed that the pathogen (*P. griseola*) did not exhibit any growth when *Trichoderma asperellum* was present. The development of *P. griseola* was entirely suppressed by all the *T. asperellum* strains tested in Petri dishes, resulting in 100% inhibition of the mycelial growth (Fig. 1). After six days of incubation, the Petri dishes were colonized entirely by all tested *Trichoderma* strains, preventing any growth of the slow-growing phytopathogen, *P. griseola*. This was observed until 45 days after incubation. In addition, *T. asperellum* grew when the pathogen was recovered from the contact zone, indicating that *P. griseola* did not survive in the presence of *Trichoderma*.

Table 1. Dual culture between *Trichoderma asperellum* 659-7, PR11, and PR12 and *Pseudocercospora griseola* after 45 days of incubation

Trichoderma asperellum strains	Inhibition rate [%]
Trichoderma asperellum 659-7	100 ± 0.00
Trichoderma asperellum PR11	100 ± 0.00
Trichoderma asperellum PR12	100 ± 0.00

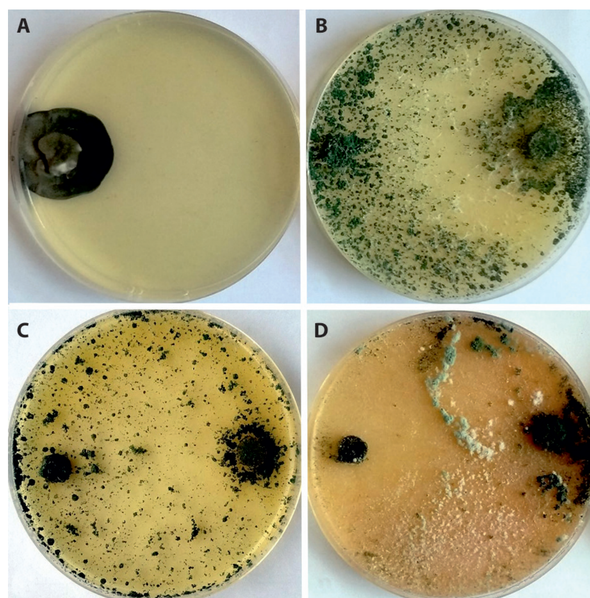

Fig. 1. Direct confrontation between *Trichoderma asperellum* PR11, PR12, 659-7 and *P. griseola* after 45 days of incubation. A – Control (*P. griseola* alone); B – dual culture between *P. griseola* and *T. asperellum* PR11; C – dual culture between *P. griseola* and *T. asperellum* PR12 and C – dual culture between *P. griseola* and *T. asperellum* 659-7

Effect of cell-free culture filtrates of *Trichoderma asperellum* 659-7, PR11 and PR12 on the mycelial growth and spore germination of *Pseudocercospora griseola*

The food poisoning technique demonstrated that the cell-free culture filtrates of all *Trichoderma asperellum* strains exhibited inhibitory effects on *P. griseola* growth (Fig. 2). The percentage of inhibition varied based on the concentration of the culture filtrate in the medium and the specific *T. asperellum* strain used. At a concentration of 12.5% of cell-free culture filtrates of *T. asperellum* 659-7, PR11and PR12, mycelial growth of *P. griseola* was inhibited by 0.8, 3.68 and 11.83%, respectively (Fig. 3). Increasing the concentration to 25% resulted in higher inhibition rates: 3.42% for 659-7, 10.01% for PR12, and 26.33% for PR11 (Fig. 3).

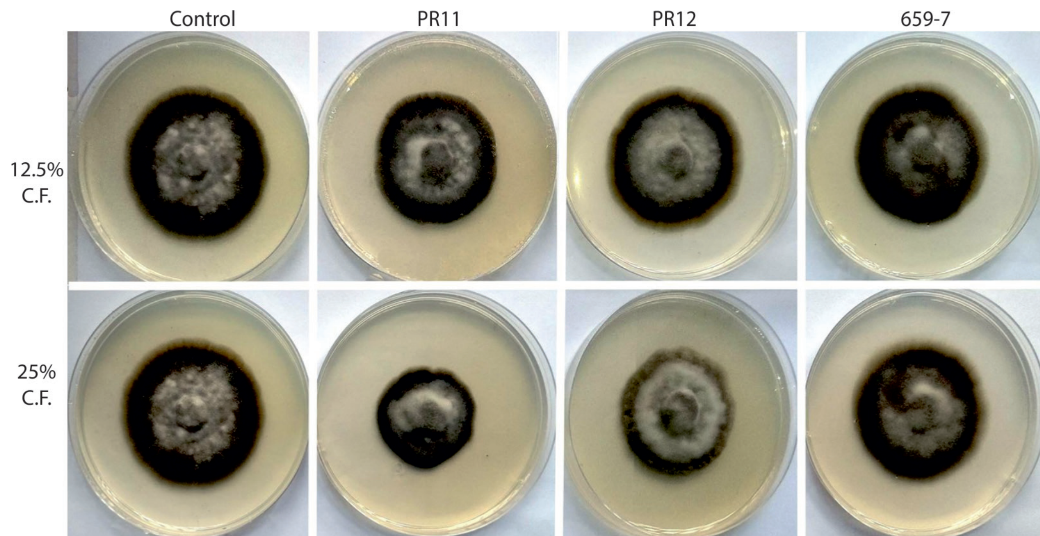

Fig. 2. Effect of different concentrations of cell-free culture filtrates (C.F) from *Trichoderma asperellum* strains (PR11, PR12 and 659-7) on the mycelial growth of *Pseudocercospora griseola*

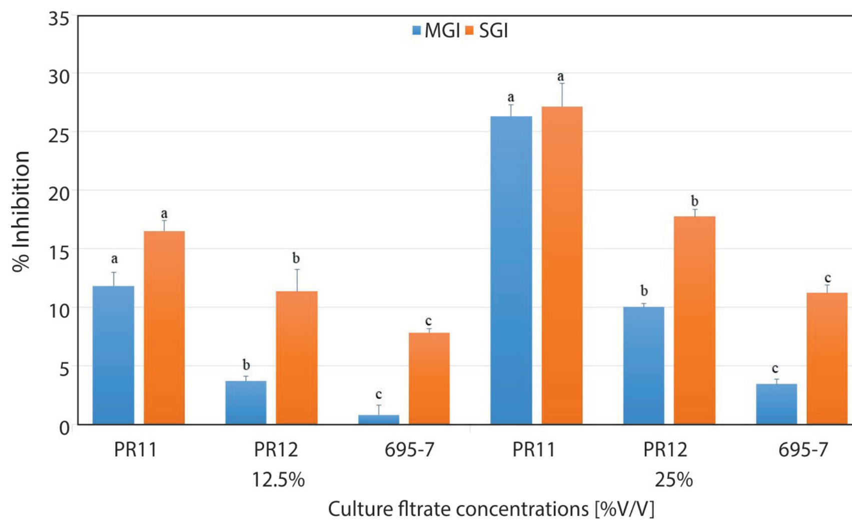

Fig. 3. Effect of different concentrations of cell-free culture filtrates (C.F) from *Trichoderma asperellum* strain PR11, PR12 and 659-7 on the mycelial growth and the spore germination of *Pseudocercospora griseola*

The process by which a fungal spore transforms from a dormant biological organism into a vegetative growing organism that can reproduce either sexually or asexually is called germination. During this process, the spore undergoes structural changes and is considered germinated once the germ tube reaches a length equal to or greater than its diameter. The effect of the cell-free culture filtrates of *Trichoderma asperellum* PR11, PR12 and 659-7 on spore germination of *P. griseola* are illustrated in Figure 3 and Figure 4. Accordingly, the percentage of inhibition of spore germination of *P. griseola* also depended on the concentration of cell-free culture filtrate and the specific strain

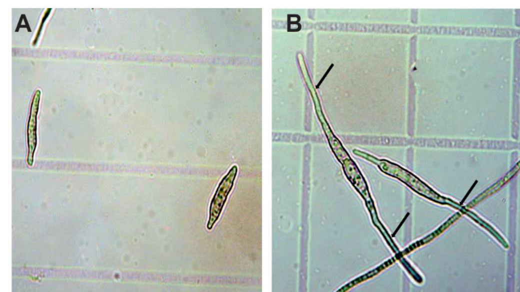

Fig. 4. Effect of cell-free culture filtrates from *Trichoderma asperellum* strain PR11, PR12 and 659-7 on the spore germination of *Pseudocercospora griseola*. Black arrows represent the germ tube. A – spore of *P. griseola*; B – germinated spore of *P. griseola*

producing this culture filtrate. At 12.5%, the highest inhibition of spore germination (16.50%) was observed with the culture filtrates of *T. asperellum* PR11, while the cell-free culture filtrates of *T. asperellum* PR12 and 659-7 inhibited spore germination by 11.37 and 7.84%, respectively (Fig. 3). When the concentration was increased to 25%, 27.14% of the *P. griseola* spores were inhibited by the culture filtrate of *T. asperellum* PR11, 17.74% by those from *T. asperellum* PR12, and 11.24% by the culture filtrate of *T. asperellum* 659-7 (Fig. 3).

In vivo evaluation of selected *Trichoderma asperellum* PR11 for the control of angular leaf spot

Bean leaves began to exhibit signs of ALS 10 days after infection by the pathogen, with symptoms becoming clearly pronounced by 14 days' post-inoculation (Fig. 5). The data in Table 2 present the effect of inoculation of bean leaves with *Pseudocercospora griseola,* both individually and alongside with *Trichoderma asperellum* PR11 on the incidence and severity of ALS 14 and 21 days after inoculation. According to Table 2, all plants infected by the pathogen developed disease during these observation periods, resulting in a 100% disease incidence, while plants treated with sterile distilled water showed no disease symptoms (0% disease incidence). However, the severity varied significantly ($p < 0.05$) depending on treatment with or without *Trichoderma asperellum* PR11. Forty days after infection with *P. griseola*, the severity of bean leaves inoculated solely with *P. griseola* was 6.36. In contrast, those treated with *T. asperellum* PR11 showed a disease severity of 5.64, indicating an 11.32% reduction in disease severity due to the application of the biocontrol agent (Table 2). On the 21st day post-inoculation, the application of *T. asperellum* PR11 resulted in a 22.5% reduction in disease severity in bean leaves inoculated with the pathogen compared to leaves infected with *P. griseola* alone. Additionally, on both the 14th and 21st day after inoculation, no symptoms were observed in the non-inoculated bean leaves, and the disease score was 1.

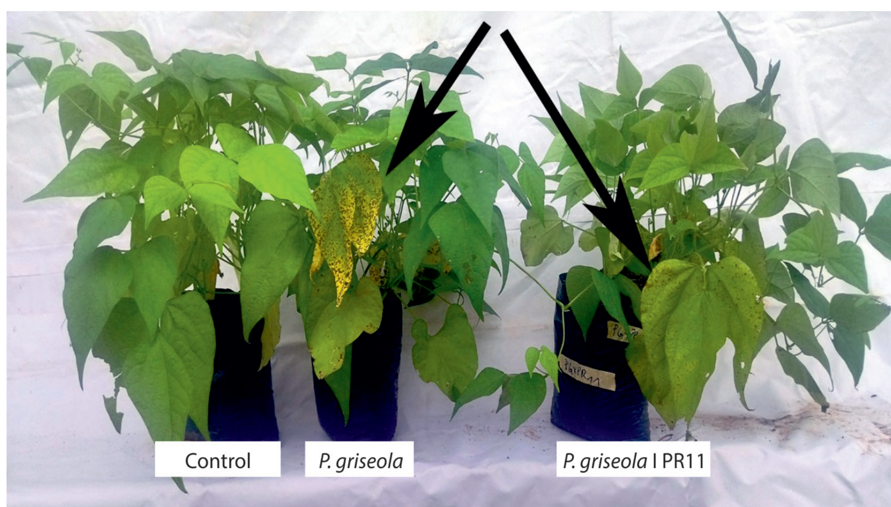

Fig. 5. Effect of single and dual inoculation of bean leaves with *Pseudocercospora griseola* and *Trichoderma asperellum* PR11 on angular leaf spot. Black arrows represent angular leaf spot lesions on bean leaves. Control: Bean leaves free from any fungal inoculation; *P. griseola*: Bean leaves infected with *P. griseola*; *P. griseola* + PR11: Bean leaves inoculated with *P. griseola* and treated with *T. asperellum* PR11

Table 2. Effect of treatment of bean leaves by *Trichoderma asperellum* PR11 on disease parameters the 14 and 21 DAI

Treatment	14 DAI			21 DAI		
	DI [%]	DS [1–9 scale]	Protection [%]	DI [%]	DS [1–9 scale]	Protection [%]
Control	0.0	1.0 ± 0.0 a	–	0.0	1.0 ± 0.0 a	–
Pg	100	6.36 ± 0.67 c	–	100	8.09 ± 0.54 c	–
Pg + PR11	100	5.64 ± 0.50 b	11.32	100	6.2 7± 0.47 b	22.5

Control: bean leaves free from any fungal inoculation; Pg: bean leaves infected by *Pseudocercospora griseola*; Pg + PR11: bean leaves inoculated with *Pseudocercospora griseola* and treated with *Trichoderma asperellum* PR11. DI = disease incidence; DS = disease severity; DAI = days after inoculation. Different letters in the same column indicate a significant difference in mean ± standard deviation values at $p \leq 0.05$

Effect of inoculation of bean leaves on some biochemical parameters

Table 3 summarizes the effect of inoculation of bean leaves with only *Pseudocercospora griseola* as well as with *Trichoderma asperellum* PR11 on the chlorophyll content, phenols and flavonoids. Compared to the control, infection of bean leaves with *P. griseola* alone resulted in an 85.238% decrease in chlorophyll, while a 42.887% reduction was noted in bean leaves infected with *P. griseola* and treated with *T. asperellum* PR11.

Table 3. Impact of single and dual inoculation of bean leaves by *Pseudocercospora griseola* and *Trichoderma asperellum* PR11 strain on the chlorophyll content, total phenols and flavonoids

Treatment	Chlorophyll [mg/g fresh weight]	Phenol [µg GAE/g dry weight]	Flavonoid [µg CE/g dry weight]
Control	0.210 ± 0.016 a	7.122 ± 1.142 c	51.241 ± 4.315 c
Pg	0.031 ± 0.008 c	18.397 ± 0.587 b	80.315 ± 3.615 b
Pg + PR11	0.120 ± 0.006 b	26.329 ± 2.198 a	138.815 ± 2.797 a

Control: Bean leaves free from any fungal inoculation; Pg: Bean leaves infected by *P. griseola*; Pg + PR11: Bean leaves inoculated with *P. griseola* and treated using the strain PR11 of *T. asperellum*. GAE – gallic acid equivalent; CE – catechin equivalents. The same superscript letter indicates that the mean ± standard deviation values of the same dependent variable do not differ significantly at $p < 0.05$

In contrast to chlorophyll content, inoculating bean leaves with the pathogen, either individually or together with the biocontrol agent, significantly increased the accumulation of phenolic compounds and flavonoids ($p < 0.05$). Untreated infected bean leaves showed a 158.42% increase in total phenolic compounds, while treated infected leaves exhibited an even greater increase of 269.80% in comparison to the control. Additionally, the flavonoid content increased by 56.73% in bean leaves infected solely with *P. griseola* and by 170.90% in those infected and treated with *T. asperellum* PR11, compared to the control.

Discussion

Angular leaf spot is a highly destructive disease that severely impacts bean quality and productivity globally (Nay *et al*. 2019). The need to develop eco-friendly and durable strategies for managing this disease led to the investigation of the biological control capability of three strains of *T. asperellum* against *P. griseola*. All the tested *T. asperellum* strains (659-7, PR11 and PR12) completely stopped *P. griseola* during the confrontation test. This result confirms the antagonistic potential of these biological control agents against plant-pathogenic fungi, aligning with research conducted by Mbarga *et al*. (2012), who showed that those strains reduced by up to 66% the mycelial growth of *Pythium myriotylum*. Wu *et al*. (2017) also characterized a novel strain of *Trichoderma asperellum* GDFS1009 and demonstrated that it reduces by 80.82% the growth of *Fusarium oxysporum* f. sp. *cucumerinum* during the antagonistic test. *Trichoderma* species are fast-growing fungi that grow rapidly and absorb the nutrients that the pathogenic fungus needs to thrive, leading to nutrient depletion and preventing the pathogenic fungus from growing and reproducing (Guo *et al*. 2019; Bazghaleh *et al*. 2020; Halifu *et al*. 2020). In addition, *Trichoderma* directly invades or wounds the mycelium of phytopathogenic fungi, resulting in changes such as growth abnormalities, shortening, rounding, protoplasmic shrinkage and breakdown of the cell membrane of the pathogen cells (Yao *et al*. 2023). These mechanisms could explain the inhibition caused by the three strains of *T. asperellum* tested.

Furthermore, the ability of *Trichoderma* to fight against plant pathogens is associated with additional mechanisms of action involving the production of antagonistic substances (El-Hasan *et al*. 2022). According to Saravanakumar *et al*. (2017), these antagonistic compounds released by *Trichoderma* spp. reduce the growth of the mycelium and inhibit the spore germination of phytopathogenic fungi. They include antibiotic substances and extracellular enzymes mainly found in their culture filtrates (Leelavathi *et al*. 2014). This could explain the results from this work, which showed that cell-free culture filtrates from *T. asperellum* 659-7, PR11, and PR12 reduced the growth of the mycelium, and inhibited the germination of spores of *P. griseola*, with percentages of inhibition varying depending on the quantity of the culture filtrate incorporated in the medium, along with the specific strain of *T. asperellum* involved. These results align with the findings of Alfiky (2019), who indicated that culture filtrates of *T. asperellum* diminish by 47.2% the mycelium development of *Rhizoctonia solani*, a fungal pathogen responsible for web blight in common bean. Raut *et al*. (2014) also observed reductions of 60 and 70.5% of the mycelium development of the same pathogen when *Trichoderma* T50 culture filtrate in the PDA medium was 10 and 25%, respectively. Wu *et al*. (2017) further demonstrated that the culture filtrate of *T. asperellum* GDFS1009 inhibits the mycelial development and the spore germination of *Fusarium* by 67.59 and 76.28%, respectively. On the other hand, data gathered by Nurbailis *et al*. (2019) indicated that culture filtrates of *Trichoderma harzianum* led to a 55.09% reduction in the conidia germination of *Colletotrichum gloeosporioides*. The noted decline in those two growth parameters of the used pathogen by the tested

T. asperellum strains might result from the antimicrobial compounds produced by *T. asperellum*. According to Druzhinina *et al*. (2018), *Trichoderma* species can generate bioactive substances that act antagonistically against phytopathogenic fungi. These bioactive compounds, which include enzymes and metabolites that break down cell walls, can successfully increase plant tolerance and decrease plant disease (Kubicek *et al.* 2019). For example, chitinase, a hydrolytic enzyme produced by *T. harzianum* inhibits both mycelial development and germination of *Fusarium graminearum* (Saravanakumar *et al*. 2017). Another enzyme found in the culture filtrates and produced by the strains that were tested is the β-1,3-glucanase (Tondje *et al*. 2007). This enzyme causes substantial destruction of the cell walls of the pathogen, thereby preventing spore germination and mycelium development (Sun *et al*. 2006). The variations in the inhibitory effect noted among the cell-free culture filtrates from the three tested *T. asperellum* strains may be because various isolates belonging to one species of *Trichoderma* produce distinct metabolites that have varying capacities to suppress the development of a pathogenic fungus (Wu *et al*. 2017).

The strain that showed the best antifungal activity during *in vitro* tests was subjected to an *in vivo* assay to assess its capacity to protect bean leaves against ALS. According to the results, the strain PR11 of *T. asperellum* decreased disease severity by 11.32 and 22.5%, respectively, at 14 and 21 days after inoculation. This outcome highlights the capacity of *T. asperellum* to fight against foliar diseases, and it aligns with the findings obtained by Jemo *et al.* (2023), who showed that soil treatment with *T. asperellum,* followed by foliar spraying of bean leaves, lowered the infection rate and the disease. The observed reduction in disease severity in treated infected bean leaves was associated with a significant increase in chlorophyll content, showcasing PR11's effectiveness in mitigating the harmful impacts of ALS. The increase in disease severity of ALS reduces the photosynthetic efficiency of the bean leaves, and therefore causes bean leaves to turn yellow due to chlorophyll shortage (Cardona *et al*. 1995; Taboada *et al*. 2022). Therefore, an increase in chlorophyll content may indicate how well *T. asperellum* PR11 protects the plant. In addition, infection of bean leaves with *P. griseola* followed by treatment with *T. asperellum* showed elevated phenolic compounds and flavonoids compared to leaves infected by the pathogen alone. This demonstrates their role in protecting the plant against the disease. In addition to mycoparasitism, antibiosis and competition, *Trichoderma* spp. can help plants fight against disease by induction of systemic defense (Asad 2022; Tyśkiewicz *et al*. 2022). During this induction, an accumulation of phenolic compounds, phenylalanine ammonia-lyase, and peroxidase activity have sometimes been observed (Ferrer *et al*. 2008; Król *et al*. 2015). This could explain the increase in total phenols, and flavonoids in infected bean leaves treated with the biocontrol agent compared to untreated infected leaves. These results align with those of Behiry *et al.* (2023), who observed an enhancement in total phenols in tomato leaves when the plant was previously inoculated with *Rhizoctonia solani* and then treated with *T. pubescens* compared to plants infected by the pathogen alone. In addition, Bala *et al*. (2024) also showed that foliar spraying of *Momordica charantia* by spore suspension of *Trichoderma* increases the accumulation of flavonoids and polyphenols by 50 and 17%, respectively. Abd El-Rahman and Mohamed (2014) found that when faba bean (*Vicia fabae* L.) leaves are infected with *Botrytis fabae* and treated with *T. harzianum*, the severity of chocolate spot disease is lowered by 41.54%, and the overall flavonoid content increases in comparison to leaves infected by *Botrytis cinerea* alone. Phenolic compounds, a particular category of secondary metabolites produced in plants, are crucial for defending the plant against both abiotic and biotic stresses. They serve as the primary defense mechanism used to fend off invasive pathogens in plants. Phenols are produced through the phenylpropanoid metabolic pathway and enhance plants' resistance by providing antimicrobial, antioxidant, and signaling functions. Certain phenols, such as phytoalexins and tannins, inhibit the growth of phytopathogens, including bacteria, fungi, and viruses, by disrupting their cell membranes or interfering with their enzymatic activities. Others, like salicylic acid, trigger systemic acquired resistance, providing resistance-enhancing to infected plants (Kumar *et al*. 2020).

Acknowledgements

This research was carried out in the Laboratory of Biological Control and Applied Microbiology of the Institute of Agricultural Research for Development (IRAD). Therefore, our sincere gratitude is extended to IRAD for providing access to their laboratory facilities. We also thank Bertrand Zing Zing and Etienne Akoutou for their assistance and support.

References

Abadio A.K.R., Lima S.S., Santana M.F., Salomão T.M.F., Sartorato A., Mizubuti E.S.G., Araújo E.F., de Queiroz M.V. 2012. Genetic diversity analysis of isolates of the fungal bean pathogen *Pseudocercospora griseola* from central and southern Brazil. Genetics and Molecular Research 11 (2): 1272–1279. DOI: http://dx.doi.org/10.4238/2012

Abd El-Rahman S.S., Mohamed H.I. 2014. Application of benzothiadiazole and *Trichoderma harzianum* to control faba bean chocolate spot disease and their effect on some physiological and biochemical traits. Acta Physiologiae Plan-

tarum 36: 343–354. DOI: https://doi.org/10.1007/s11738-013-1416-5
Abeysinghe S. 2009. Systemic resistance induced by *Trichoderma harzianum* RU01 against *Uromyces appendiculatus* on *Phaseolus vulgaris*. Journal of the National Science Foundation of Sri Lanka 37 (3): 203. DOI: http://dx.doi.org/10.4038/jnsfsr.v37i3.1214
Adomako J., Yeboah S., Asamoah J.F., Amankwaa-Yeboah P., Adjei E.A., Obeng E.A., Sakyiamah B., Lamptey M., Butare L., Asibuo J.Y. 2022. Survey of plant parasitic nematodes and disease severity of common bean lines evaluated for reaction to root knot nematodes infestation. African Crop Science Journal 30 (2): 147–154. DOI: https://doi.org/10.4314/acsj.v30i2.3
Alfiky A. 2019. Effects of ultraviolet irradiation on the *in vitro* antagonistic potential of *Trichoderma* spp. against soil-borne fungal pathogens. Heliyon 5 (7): e02111. DOI: https://doi.org/10.1016/j.heliyon.2019.e02111
Arnon D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24 (1): 1–15. DOI: https://doi.org/10.1104/pp.24.1.1
Asad S.A. 2022. Mechanisms of action and biocontrol potential of *Trichoderma* against fungal plant diseases – A review. Ecological Complexity 49: 100978. DOI: https://doi.org/10.1016/j.ecocom.2021.100978
Bala I.A., Șesan T.E., Oancea A., Craciunescu O., Ghiurea M., Răut I., Trică B., Nicolae C.-A., Constantinescu-Aruxandei D., Oancea F. 2024. Influence of foliar treatment with suspensions rich in *Trichoderma chlamydospores* on *Momordica charantia* physiology, yield, and quality. Horticulturae 10 (4): 371. DOI: https://doi.org/10.3390/horticulturae10040371
Bazghaleh N., Prashar P., Woo S., Vandenberg A. 2020. Effects of lentil genotype on the colonization of beneficial *Trichoderma* species and biocontrol of Aphanomyces root rot. Microorganisms 8 (9): 1290. DOI: https://doi.org/10.3390/microorganisms8091290
Begoude B.A.D., Lahlali R., Friel D., Tondje P.R., Jijakli M.H. 2007. Response surface methodology study of the combined effects of temperature, pH, and aw on the growth rate of *Trichoderma asperellum*. Journal of Applied Microbiology 103 (4): 845–54. DOI: http://dx.doi.org/10.1111/j.1365-2672.2007.03305.x
Behiry S., Soliman S.A., Massoud M.A., Abdelbary M., Kordy A.M., Abdelkhalek A., Heflish A. 2023. *Trichoderma pubescens* elicit induced systemic resistance in tomato challenged by *Rhizoctonia solani*. Journal of Fungi 9 (2): 167. DOI: https://doi.org/10.3390/jof9020167
Benítez T., Rincón A.M., Limón M.C., Codon A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology 7: 249–260.
Bi M.E., Annih M.G., Mboh K.S., Kinge T.R. 2023. Effect of angular leaf spot disease on bean inter-cropped with maize in Foumbot and Bambili, Cameroon. Journal of the Cameroon Academy of Sciences 19: 209–220.
Broughton W.J., Hernández G., Blair M., Beebe S., Gepts P., Vanderleyden J. 2003. Beans (*Phaseolus* spp.) – model food legumes. Plant and Soil 252 (1): 55–128. DOI: https://doi.org/10.1023/A:1024146710611
Busby P.E., Ridout M., Newcombe G. 2016. Fungal endophytes: modifiers of plant disease. Plant Molecular Biology 90 (6): 645–655. DOI: https://doi.org/10.1007/s11103-015-0412-0
Cajiao C., Jara C., Beebe S., Mosquera G. 2023. Rating scale for field evaluations of common bean genotypes (accessions, advanced lines, or varieties) using artificial inoculation. CIAT Publication No. 542, Cali, Colombia, 26 pp.
Cardona C., Flor C.A., Morales F., Pastor-Corrales M.A. 1995. Problemas de campo en los cultivos de frijol en el trópico. International Center for Tropical Agriculture (CIAT), Cali, Colombia, 1–5.
Castellamos G., Jara C., Mosquera G. 2016. Bean pathogens: practical guide for Lab and greenhouse work. 2nd ed. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 257 pp.
Comporta P. 1985. Antagonisme *in vitro* de *Trichoderma* spp. vis-à-vis de *Rhizoctonia solani* Kuhn. Agronomies 5: 613–620. DOI: https://doi.org/10.1051/agro:19850707
Crous P.W., Liebenberg M.M., Braun U., Groenewald J.Z. 2006. Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean. Studies in Mycology 55: 163–173. DOI: https://doi.org/10.3114/sim.55.1.163
Ddamulira G. 2019. Pathogen variability and new sources of resistance to angular leaf spot among bean landraces in Uganda. African Journal of Food, Agriculture, Nutrition and Development 19 (01): 13905–13927. DOI: https://doi.org/10.18697/ajfand.84.blfb1006
Ddamulira G., Mukankusi C., Ochwo-Ssemakula M., Edema R., Sseruwagi P., Gepts P. 2014. Distribution and variability of *Pseudocercospora griseola* in Uganda. Journal of Agricultural Science 6 (6). DOI: https://doi.org/10.5539/jas.v6n6p16
Dewanto V., Wu X., Adom K.K., Liu R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50 (10): 3010–3014. DOI: https://doi.org/10.1021/jf0115589
Druzhinina I.S., Chenthamara K., Zhang J., Atanasova L., Yang D., Miao Y., Rahimi M.J., Grujic M., Cai F., Pourmehdi S. 2018. Massive lateral transfer of genes encoding plant cell wall-degrading enzymes to the mycoparasitic fungus *Trichoderma* from its plant-associated hosts. PLOS Genetics 14: e1007322.
Eke P., Wakam L.N., Fokou P.V.T., Ekounda T.V., Sahu K.P., Wankeu T.H.K., Boyom F.F. 2019. Improved nutrient status and *Fusarium* root rot mitigation with an inoculant of two biocontrol fungi in the common bean (*Phaseolus vulgaris* L.). Rhizosphere 12: 100172.
El-Hasan A., Walker F., Klaiber I., Schöne J., Pfannstiel J., Voegele R.T. 2022. New approaches to manage Asian soybean rust (*Phakopsora pachyrhizi*) using *Trichoderma* spp. or their antifungal secondary metabolites. Metabolites 12 (6): 507. DOI: https://doi.org/10.3390/metabo12060507
FAO. Food and Agriculture Organization of the United Nations Data-base 2023. [Available on: https://www.fao.org/faostat].
Ferreira F.V., Musumeci M.A. 2021. *Trichoderma* as biological control agent: scope and prospects to improve efficacy. World Journal of Microbiology and Biotechnology 37 (5). DOI: https://doi.org/10.1007/s11274-021-03058-7
Ferrer J.-L., Austin M.B., Stewart Jr C., Noel J.P. 2008. Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiology and Biochemistry 46 (3): 356–370. DOI: https://doi.org/10.1016/j.plaphy.2007.12.009
Gael C.C., Pierre E., Hortense A.M., Christian D.T., Victorine L., Felix F., Christopher S., Aime Didier B.B., Louise W.N., Fabrice F.B. 2025. Occurrence and characterization of *Pseudocercospora griseola*, the causative agent of common bean angular leaf spot in the Western Highlands agro-ecological zone of Cameroon. Archives of Phytopathology and Plant Protection 58 (14): 811–828. DOI: http://dx.doi.org/10.1080/03235408.2025.2533446
Global Dry Beans Production by Country 2023. In: ReportLinker. https://www.reportlinker.com/dataset/a8944c4bfe41484f9e07653394553894a041507. [Accessed 7 May 2025].
Grover R.K., Moore, J.D. 1962. Toximetric Studies of Fungicides against the Brown Rot Organisms, *Sclerotinia fructicola* and *S. laxa*. Phytopathology 52: 876-879.
Guo Y., Ghirardo A., Weber B., Schnitzler J.-P., Benz J.P., Rosenkranz M. 2019. *Trichoderma* species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor.* Frontiers in Microbiology 10. DOI: https://doi.org/10.3389/fmicb.2019.00891
Halifu S., Deng X., Song X., Song R., Liang X. 2020. Inhibitory mechanism of *Trichoderma virens* ZT05 on *Rhizocto-*

nia solani. Plants 9 (7): 912. DOI: https://doi.org/10.3390/plants9070912

Hyde K.D., Xu J., Rapior S., Jeewon R., Lumyong S., Niego A.G.T., Abeywickrama P.D., Aluthmuhandiram J.V.S., Brahamanage R.S., Brooks S., Chaiyasen A., Chethana K.W.T., Chomnunti P., Chepkirui C., Chuankid B., De Silva N.I., Doilom M., Faulds C., Gentekaki E., Gopalan V., Kakumyan P., Harishchandra D., Hemachandran H., Hongsanan S., Karunarathna A., Karunarathna S.C., Khan S., Kumla J., Jayawardena R.S., Liu J.-K., Liu N., Luangharn T., Macabeo A.P.G., Marasinghe D.S., Meeks D., Mortimer P.E., Mueller P., Nadir S., Nataraja K.N., Nontachaiyapoom S., O'Brien M., Penkhrue W., Phukhamsakda C., Ramanan U.S., Rathnayaka A.R., Sadaba R.B., Sandargo B., Samarakoon B.C., Tennakoon D.S., Siva R., Sriprom W., Suryanarayanan T.S., Sujarit K., Suwannarach N., Suwunwong T., Thongbai B., Thongklang N., Wei D., Wijesinghe S.N., Winiski J., Yan J., Yasanthika E., Stadler M. 2019. The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity 97 (1): 1–136. DOI: https://doi.org/10.1007/s13225-019-00430-9

Jemo M., Nkenmegne S., Buernor A.B., Raklami A., Ambang Z., Souleyamanou A., Ouhdouch Y., Hafidi M. 2023. Mycorrhizas and *Trichoderma* fungi increase the accumulation of secondary metabolites in grain legume leaves and suppress foliar diseases in field-grown conditions of the humid forest of Cameroon. BMC Plant Biology 23 (1). DOI: https://doi.org/10.1186/s12870-023-04587-z

Karavidas I., Ntatsi G., Vougeleka V., Karkanis A., Ntanasi T., Saitanis C., Agathokleous E., Ropokis A., Sabatino L., Tran F. 2022. Agronomic practices to increase the yield and quality of common bean (*Phaseolus vulgaris* L.): A systematic review. Agronomy 12 (2): 271. DOI: https://doi.org/10.3390/agronomy12020271

Kebede E. 2021. Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. Frontiers in Sustainable Food Systems 5. DOI: https://doi.org/10.3389/fsufs.2021.767998

Król P., Igielski R., Pollmann S., Kępczyńska E. 2015. Priming of seeds with methyl jasmonate induced resistance to hemibiotroph *Fusarium oxysporum* f. sp. *lycopersici* in tomato via 12-oxo-phytodienoic acid, salicylic acid, and flavonol accumulation. Journal of Plant Physiology 179: 122–132.

Kubicek C.P., Steindorff A.S., Chenthamara K., Manganiello G., Henrissat B., Zhang J., Cai F., Kopchinskiy A.G., Kubicek E.M., Kuo A., Baroncelli R., Sarrocco S., Noronha E.F., Vannacci G., Shen Q., Grigoriev I.V., Druzhinina I.S. 2019. Evolution and comparative genomics of the most common *Trichoderma* species. BMC Genomics 20 (1). DOI: https://doi.org/10.1186/s12864-019-5680-7

Kumar S., Abedin M.M., Singh A.K., Das S. 2020. Role of Phenolic Compounds in Plant-Defensive Mechanisms. p. 517–532. In: " Plant Phenolics in Sustainable Agriculture" (Lone R., Shuab R., Kamili, A., eds.). Springer, Singapore, 596 pp.

Leelavathi M.S., Vani L., Reena P. 2014. Antimicrobial activity of *Trichoderma harzianum* against bacteria and fungi. International Journal of Current Microbiology and Applied Sciences 3: 96–103.

Mayo-Prieto S., Campelo M.P., Lorenzana A., Rodríguez--González A., Reinoso B., Gutiérrez S., Casquero P.A. 2020. Antifungal activity and bean growth promotion of *Trichoderma* strains isolated from seed vs soil. European Journal of Plant Pathology 158 (4): 817–828. DOI: https://doi.org/10.1007/s10658-020-02069-8

Mbarga J.B., Ten Hoopen G.M., Kuaté J., Adiobo A., Ngonkeu M.E.L., Ambang Z., Akoa A., Tondje P.R., Begoude B.A.D. 2012. *Trichoderma asperellum*: A potential biocontrol agent for *Pythium myriotylum*, causal agent of cocoyam (*Xanthosoma sagittifolium*) root rot disease in Cameroon. Crop Protection 36: 18–22.

Mohiddin F.A., Khan M.R., Khan S.M., Bhat B.H. 2010. Why *Trichoderma* is considered super hero (super fungus) against the evil parasites? Plant Pathology Journal 9 (3): 92–102. DOI: https://doi.org/10.3923/ppj.2010.92.102

Mokhtar H., Aid D. 2013. Contribution in isolation and identification of some pathogenic fungi from wheat seeds, and evaluation of antagonistic capability of *Trichoderma harzianum* against those isolated fungi *in vitro*. Agriculture and Biology Journal of North America 4: 145–154.

Mujica M.V., Granito M., Soto N. 2009. Importance of the extraction method in the quantification of total phenolic compounds in *Phaseolus vulgaris* L. Interciencia 34: 650–654.

Nay M.M., Souza T.L.P.O., Raatz B., Mukankusi C.M., Gonçalves-Vidigal M.C., Abreu A.F.B., Melo L.C., Pastor-Corrales M.A. 2019. A Review of angular leaf spot resistance in common bean. Crop Science 59: 1376–1391. DOI: https://doi.org/10.2135/cropsci2018.09.0596

Nchanji E.B., Ngoh S.B., Toywa J., Cosmas L. 2023. Analysis of common bean (*Phaseolus vulgaris* L.) trade in Cameroon: A trader's perspective of preferred varieties and market traits. Journal of Agriculture and Food Research 14: 100839. DOI: https://doi.org/10.1016/j.jafr.2023.100839

Nurbailis N., Djamaan A., Rahma H., Liswarni Y. 2019. Potential of culture filtrate from *Trichoderma* spp. as biofungicide to *Colletotrichum gloeosporioides* causing anthracnose disease in chili. Biodiversitas Journal of Biological Diversity 20 (10). DOI: https://doi.org/10.13057/biodiv/d201020

Nutter J., Teng P.S., Shokes F.M. 1991. Disease assessment terms and concepts. Plant Disease 75: 1187–1188

O'Brien P.A. 2017. Biological control of plant diseases. Australasian Plant Pathology 46: 293–304. DOI: https://doi.org/10.1007/s13313-017-0481-4

Palacıoğlu G., Özer G., Yeken M.Z., Çiftçi V., Bayraktar H. 2021. Resistance sources and reactions of common bean (*Phaseolus vulgaris* L.) cultivars in Turkey to anthracnose disease. Genetic Resources and Crop Evolution 68 (8): 3373–3381. DOI: https://doi.org/10.1007/s10722-021-01195-4

Papathanasiou F., Ninou E., Mylonas I., Baxevanos D., Papadopoulou F., Avdikos I., Sistanis I., Koskosidis A., Vlachostergios D.N., Stefanou S., Tigka E., Kargiotidou A. 2022. The Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes under water stress based on physiological and agronomic parameters. Plants 11: 2432. DOI: https://doi.org/10.3390/plants11182432

Pastor-Corrales M.A., Jara C., Singh S.P. 1998. Pathogenic variation in, sources of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. Euphytica 103 (2): 161–171. DOI: https://doi.org/10.1023/a:1018350826591.

Phoka N., Suwannarach N., Lumyong S., Ito S., Matsui K., Arikit S., Sunpapao A. 2020. Role of volatiles from the endophytic fungus *Trichoderma asperelloides* PSU-P1 in biocontrol potential and in promoting the plant growth of *Arabidopsis thaliana*. Journal of Fungi 6 (4): 341. DOI: https://doi.org/10.3390/jof6040341

Poveda J., Abril-Urias P., Escobar C. 2020. Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi. Frontiers in Microbiology 11. DOI: https://doi.org/10.3389/fmicb.2020.00992

Punja Z.K., Utkhede R.S. 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends in Biotechnology 21 (9): 400–407. DOI: https://doi.org/10.1016/s0167-7799(03)00193-8

Raut I., Badea-Doni M., Calin M., Oancea F., Vasilescu G., Sesan T.E., Jecu L. 2014. Effect of volatile and non-volatile metabolites from *Trichoderma* spp. against important phytopathogens. Revista de Chimie 65: 1285–1288.

Sánchez-Montesinos B., Diánez F., Moreno-Gavíra A., Gea F.J., Santos M. 2020. Role of *Trichoderma aggressivum* f. *europaeum* as plant-growth promoter in horticulture. Agronomy 10 (7): 1004. DOI: https://doi.org/10.3390/agronomy10071004

Saravanakumar K., Li Y., Yu C., Wang Q., Wang M., Sun J., Gao J., Chen J. 2017. Effect of *Trichoderma harzianum*

on maize rhizosphere microbiome and biocontrol of *Fusarium* stalk rot. Scientific Reports 7 (1). DOI: https://doi.org/10.1038/s41598-017-01680-w

Sartorato A. 2004. Pathogenic variability and genetic diversity of *Phaeoisariopsis griseola* isolates from two counties in the state of Goias, Brazil. Journal of Phytopathology 152: 385–390. DOI: https://doi.org/10.1111/j.1439-0434.2004.00858.x

Singleton V.L., Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16 (3): 144–158. DOI: https://doi.org/10.5344/ajev.1965.16.3.144

Sreedevi B., Charitha Devi M., Saigopal D.V.R. 2011. Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina*. Journal of Agricultural Technology 7: 623–635.

Sun H., Yang J., Lin C., Huang X., Xing R., Zhang K.-Q. 2006. Purification and properties of a β-1,3-glucanase from *Chaetomium* sp. that is involved in mycoparasitism. Biotechnology Letters 28: 131–135. DOI: https://doi.org/10.1007/s10529-005-5132-0

Taboada G., Abán C.L., Mercado Cárdenas G., Spedaletti Y., Aparicio González M., Maita E., Ortega-Baes P., Galván M. 2022. Characterization of fungal pathogens and germplasm screening for disease resistance in the main production area of the common bean in Argentina. Frontiers in Plant Science 13. DOI: https://doi.org/10.3389/fpls.2022.986247

Tchameni S.N., Sameza M.L., O'donovan A., Fokom R., Mangaptche Ngonkeu E.L., Wakam Nana L., Etoa F.-X., Nwaga D. 2017. Antagonism of *Trichoderma asperellum* against *Phytophthora megakarya* and its potential to promote cacao growth and induce biochemical defence. Mycology 8: 84–92. DOI: https://doi.org/10.1080/21501203.2017.1300199

Toghueo R.M.K., Eke P., Zabalgogeazcoa Í., de Aldana B.R.V., Nana L.W., Boyom F.F. 2016. Biocontrol and growth enhancement potential of two endophytic *Trichoderma* spp. from *Terminalia catappa* against the causative agent of common bean root rot (*Fusarium solani*). Biological Control 96: 8–20.

Tondje P.R., Roberts D.P., Bon M.C., Widmer T., Samuels G.J., Ismaiel A., Begoude A.D., Tchana T., Nyemb-Tshomb E., Ndoumbe-Nkeng M. 2007. Isolation and identification of mycoparasitic isolates of *Trichoderma asperellum* with potential for suppression of black pod disease of cacao in Cameroon. Biological Control 43: 202–212.

Tyśkiewicz R., Nowak A., Ozimek E., Jaroszuk-Ściseł J. 2022. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. International Journal of Molecular Sciences 23 (4): 2329. DOI: https://doi.org/10.3390/ijms23042329

Van Schoonhoven A., Pastor-Corrales M.A. 1987. Standard system for the evaluation of bean germplasm. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 53 pp.

Wani A.A., Bhat F.A., Nisa R.T., Wani T.A., Raja W., Jan S.K., Rather T.R., Anjum N., Wani M.A. Mohammad P. 2022. Bean angular leaf spot (*Phaeoisariopsis griseola*): Epidemiology and yield loss assessment. The Pharma Innovation Journal 11: 291–297.

Wu Q., Sun R., Ni M., Yu J., Li Y., Yu C., Dou K., Ren J., Chen J. 2017. Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. PLOS One 12 (6): e0179957. DOI: https://doi.org/10.1371/journal.pone.0179957

X., Guo H., Zhang K., Zhao M., Ruan J., Chen J. 2023. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Frontiers in Microbiology 14. DOI: https://doi.org/10.3389/fmicb.2023.1160551