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Full Length Research Paper

Trichoderma Isolates from the Faba bean (Vicia faba L.) Rhizosphere for their Antagonistic Potential against Botrytis fabae Sard and Plant Growth-promoting Properties: An in vitro study

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Abstract

Trichoderma is a fungus used as a biological control agent for plant disease. Chocolate spot disease is one of the fungal diseases caused by Botrytis fabae Sard in faba beans. Thus, the present study was conducted to characterize Trichoderma isolates for their antagonistic efficiency against B. fabae and plant growth-promoting traits (PGPts). The effectiveness of the isolates against B. fabae varied from 7% to 88% upon 3-9 days of incubation (DOI) using the dual culture technique. Isolates AAUT21 and AAUT14 were the most effective, with 58-88 % of inhibition. The inhibition increased from 3-9th DOI from 13-64 % by sealed plate method. In the detached leaf assay, AAUT14 and AAUT44 showed significant differences from the controls ($P < 0.05$). Moreover, 63% and 95 % of the isolates were positive for indole-3-acetic acid and ammonia synthesis, respectively. In addition, the isolates solubilized tricalcium phosphate (TCP) in the range of 135-575 µg mL^{-1} upon 3-6 DOI. A negative correlation was also observed between pH and TCP solubilization ($r = -.612^{**}$). Based on the cultural characteristics and tef1α gene sequence analysis, AAUT21, AAUT14, AAUT44, AAUT45, AAUT6, AAUT30, and AAUT4 were identified as T. afroharzianum AAUT21, T. harzianum AAUT14, T. tomentosum AAUT44, T. afroharzianum AAUT45, T. harzianum AAUT6, T. afroharzianum AAUT30, and T. tomentosum AAUT4, respectively. Trichoderma harzianum AAUT14 showed the best antagonistic feature against B. fabae along with PGPts production. Thus, T. harzianum AAUT14 can be a candidate to be used as a biofungicide for B. fabae and growth-promoting agent for faba bean plants.

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1. Introduction

Chocolate spot disease is one of the emerging fungal diseases caused by *Botrytis fabae,* with a wide host range of vegetables and legumes. The disease is widely distributed in Tunisia, Algeria, Morocco, Libya, Ethiopia, and other countries such as Spain, Norway, Germany, Scotland, Russia, Japan, China, Canada, and Australia (Hebblethwaite 1983). In Ethiopia, yield loss of up to 67.5% were recorded in susceptible faba bean cultivars by chocolate spot disease (Sahile *et al.,* 2010). Several studies showed that the disease can be managed through an integrated approach, including careful selection of seeds, the use of resistant varieties, and applications of fungicides such as MORE 720 WP (Mancozeb+Cymoxanil) and ORZEB 80WP (Mancozeb) (Kora *et al*., 2017; Tegegn *et al*., 2019).

Even though fungicides are effective in controlling fungal pathogens of plants, they induce resistance to the pathogen (Hassan *et al*., 2006; Jin and Khalid, 2022). Resistance problems could be much worse. All types of fungicide are still effective in many situations. Current countermeasures are by no means perfect, but they have proved to be necessary and beneficial (Oliver, 2021). In order to minimize the use of synthetic fungicides, selected microorganisms can be used as part of the integrated disease management (IDM). *Trichoderma* species are cosmopolitan fungi that are present in all types of soil and other habitats (wood decay, barks, etc.). *Trichoderma harzianum*, *T. koningii*, *T. orientale*, *T. tomentosum,* and *T. viride* are usually found in cellulose-rich soil and other environments (Jang *et al*., 2017). These fungi are good biological control agents (BCAs) of various aerial, root, and seed diseases of economically important crops (Galarza *et al*., 2015; Reddy *et al*., 2018). The fungi are effective in controlling phytopathogens due to their ability to grow toward the hyphae of other fungi, coil around them, and degrade the cell wall of the pathogen (mycoparasitism). Elad (2000) has reported that about 90% of different strains of *Trichoderma* spp. are being used as BCAs. Although studies on the antagonistic properties of *Trichoderma* spp., *T. harzianum*, *T. viride,* and *T. virens* on different fungal diseases showed disease reduction of 24-98% under greenhouse and field conditions on vegetables (Bokhari and Perveen, 2012; Kator *et al*., 2015), chickpeas (Yadav *et al*., 2011; Subhani *et al*., 2013), and potato blight (Al-Mughrabi, 2008; Quiroga-Rojas *et al*., 2012). However, there is limited report on chocolate spot disease on faba beans.

In addition, *Trichoderma* spp. are capable of producing ammonia and hydrogen cyanide to suppress the efficiency of pathogens to infect pathogens (Keszler *et al*., 2000) and acquire multiple plant growth-promoting properties that involve phosphate solubilization and phytohormone production. According to Reino et al. (2008), *T. harzianum, T. hamatum*, *T. asperellum,* and *T. atroviride* are commercialized for the control of phytopathogens and plant growth promoters in agriculture. To that end, some studies showed the antagonistic fungi *T. album* (Barakat et al., 2014), *Trichoderma* spp. Teshome et al. (2013) reduced the disease incidence of chocolate spot by 78 and 51 %, respectively; Mbazia et al. (2016) also showed a 35 % reduction in chocolate spot infestation on leaves of faba beans by *T. viride.* The application of *Trichoderma* spp. showed an antagonistic efficacy ranging from 47.6 to 98 % against *B. fabae* (Sahile *et al*., 2011). Mbazia et al. (2016) have reported a 35% reduction in chocolate spot infestation on leaves of faba beans by *T. viride*. Moreover, *Trichoderma* species possess a natural resistance to many chemicals and fungicides used in agriculture, therefore, they are readily integrated into agricultural practices (Ons *et al*., 2020). Thus, the objective of this study was to

isolate, screen, identify, and characterize *Trichoderma* spp. for their multiple plant growth-promoting and antagonistic effectiveness against *B. fabae*, the causative agent of chocolate spot in faba bean in Ethiopia.

2. Materials and Methods

2.1 Description of the study area Study area and sample collection

This study was conducted at Arsi zone (DigelunaTijo and Tiyo districts) and Bale zone (Goba district) as shown below (Figure 1). DigelunaTijo is located at geographical coordinates of 7° 19' 60.00" N latitude and 39° 14' 60.00" E longitude and altitude between 2500-3560 meters above sea level (a.s.l). The district is found in Arsi zone, bordered by Bekoji on the South, Munesa on the South-West, Tiyo on the North-West, Hitosa on the North, Tena on the North-East and Sherka on the East. It has 39.5% arable, 27.4% pasture, 13.3% forest and 19.8% as swampy land (SEP, 2019). Tiyo district is found at 7° 49' 59.99" N latitude and 39° 09' 60.00" E longitudes, an altitude of 1780-3100 m.a.s.l. The district is bordered by Munesa to the south, Ziway Dugda to the west, Hitosa to the northeast, and the Digeluna Tijo district in the southeastern part. This district has 40% arable land, 23.1% pasture, 8.7% forest, and 28.2% swampy land (SEP, 2019) and an altitude of 1780-3100 m. a. s. l., with a temperature of 5-28°C and relative humidity of 43-60%. Both Digeluna Tijo and Tiyo districts have bimodal rainfall, having March to April short and July to October long rainy seasons.

The Goba district is found in the Oromia region, Bale zone. It is bordered on the south by Mennana Harena Buluk, on the west by Arsi, on the north by the Mena river, and on the southeast by Berbere town with coordinates of 6° 49' 59.99" N latitude and 39° 49' 59.99" E longitude. This district is located at a distance of 14km south of Bale zonal town, Robe, and 444km southeast of Addis Ababa, with coordinates of 6° 49' 59.99" N latitude and 39° 49' 59.99" E longitude. The altitude of this district is 1500-4377 m. a. s. l., having a temperature of 0-23°C (BZMED, 2007). As a part of the Bale Zone, Goba District has two types of rainfall regimes. The long rainy season extends from March to April, with high rainfall during June, July, and August. The second rainy season of rainfall regime is influenced by equatorial westerly and easterly winds with rainfall during spring and autumn. A survey of the land in this district shows that 13% is arable or cultivable, 27.6% is pasture, 54.6% is forest (or part of the Bale Mountains National Park), and the remaining 4.8% is considered degraded or otherwise unusable (BOARD, 2012).

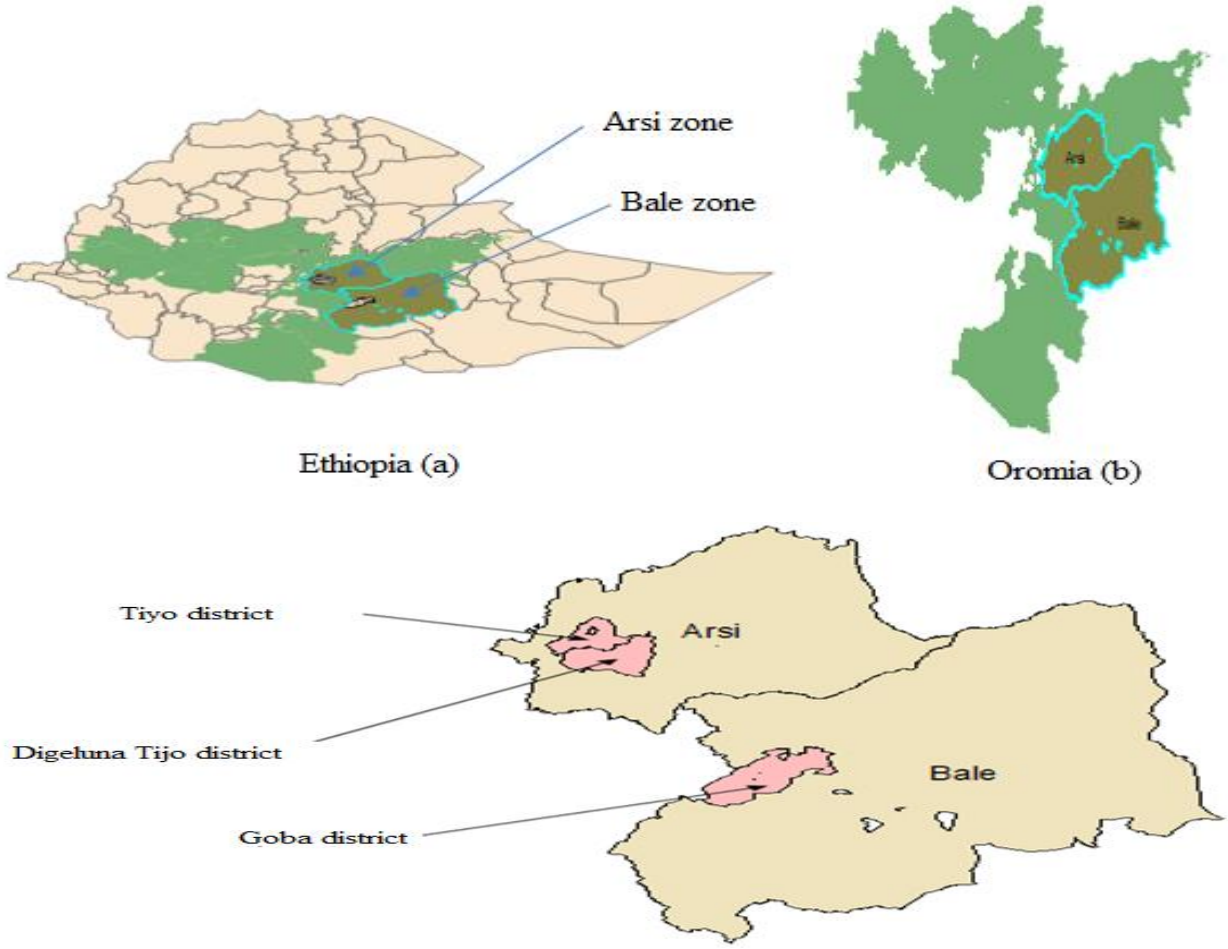


Figure 1. A map showing the study zones and selected districts (Firdu *et al*., 2022)

2.2 Soil sample collection and processing

Rhizospheric soil samples were collected from the study areas during the faba bean growing season of 2016. From each sampling site, 50 g of root-adhering soil samples were collected from the standing faba bean plants in triplicate, and composited into one and collected in alcohol-surface-sterilized polyethylene bags and stored at 4°C until the isolation of *Trichoderma* isolates.

2.3 Isolation and cultural characterization of Trichoderma isolates

The *Trichoderma* fungi were isolated according to Elad et al. (1980); 10 g of each soil sample was dissolved in 90 mL of sterilized-water in a 250 mL flask. The suspension was prepared to appropriate dilutions from which 0.1 mL of the final dilution was transferred onto Rose Bengal agar (RBA) medium ($MgSO_4.7H_2O$, 0.20 g/L, K_2HPO_4, 0.9 g/L, KCl, 0.15 g/L, NH_4NO_3, 1 g/L, glucose, 3 g/L, rose Bengal, 0.15 g/L, and agar, 20 g/L). The plates were incubated at 25±2°C for 7-10 days. Typical *Trichoderma*-resembling colonies were picked according to Barnnet et al. (1972) and subcultured on potato dextrose agar (PDA) plates for purification and characterization. The purified cultures were then compared with the characteristics and illustrations in the manual to confirm the isolates and stored in PDA slants at 4°C for further study.

2.4 Isolation of Botrytis fabae (the test pathogen)

The test fungus was isolated from leaves (showing symptoms of chocolate spot) of faba bean collected from the study areas and seeds from storage, according to Shinde (2016), using faba bean dextrose agar (FDA) medium (200 g faba bean seed extracts, 20 g dextrose, and 18 g agar). Seeds and leaves were washed with tap water, surface sterilized with 3 % sodium hypochlorite for 5 minutes and rinsed in sterilized-distilled water and air-dried. Four to six seeds and leaf cuts per plate were transferred to FDA medium and incubated at room temperature for 10-12 days under 12hr light and 12hr dark conditions, and purification was done by subculturing on PDA plates. The identification of *B. fabae* was conducted through looking at the cultural characteristics (i.e., colony color and formation of sclerotia on the PDA). The purified culture was then preserved at 4°C using PDA slants for further experimental work.

2.5 Pathogenecity test (Koch's postulate)

The pathogenecity test was done according to Abdel-Aleem et al. (2011). Faba bean seeds were surface sterilized with 3 % sodium hypochlorite for 5 min and rinsed with sterilized-distilled water. Faba bean seeds were sown in alcohol-sterilized pots filled with 3 kg of sterile soil. Fifteen days after planting, a 12 mL spore suspension having $4.5X10^5$ spores/mL was sprayed on the foliar parts of faba bean seedlings and covered with surface-sterilized plastics, and the control plants were sprayed with 12 mL sterilized water. Samples of the infected plants were utilized to re-isolate the pathogen after the 7^{th} days of spraying to confirm Koch's postulate. The isolates were tested for cultural characteristics and compared with the reference strain of *B. fabae* obtained from the Phytopathology Laboratory of the Ethiopian Institute of Agricultural Research.

2.6 Molecular identification of the isolates

2.6.1 Extraction of Deoxyribonucleic acid (DNA)

The DNA was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). Three hundred milligrams (300 mg) of mycelia mat were transferred to a sterile pestle and mortar and grounded using lysis buffer (100 mM Tris HCl [pH8.0], 50 mM EDTA, 3 % SDS), and transferred to 1.5 mL Eppendorff, with the addition of CTAB buffer, centrifuged at 13,000 rpm for 10 min. The supernatant was then mixed with 210 mg/L of RNase A and incubated at 37°C for 15 min, to which chloroform: Isoamyl alcohol (25:24:1) was added and centrifuged at 13,000 rpm for 10 min. The upper aqueous layer was treated with 100 % cold ethanol, precipitated at -20°C for 30 min, and centrifuged at 12,000 rpm for 10 min. The DNA pellet was washed with 70 % ethanol and centrifuged at 12,000 rpm for 5

min. The DNA pellets were air-dried and suspended in a 1×TE buffer. For PCR reactions, 1 µL of the extracted DNA was utilized as a template for the amplification.

2.6.2 Quantity and quality determination of DNA

The quantity of the extracted genomic DNA was determined by measuring the absorbance at 260 nm using Thermo Scientific NanoDrop (NanoDrop 2000). The quality and suitability for PCR reaction of the DNA were checked by visual comparison with the standard DNA size markers after electrophoresis through 0.8 % agarose TAE (Tris-acetate EDTA) gels stained with 0.5 mg mL^{-1} ethidium bromide (Sigma Chemicals Co.).

2.6.3 Primers and PCR conditions for DNA (Tef1α) gene amplification

The amplification of the 1.3 kb translation elongation factor 1 alpha (tef1α) gene was performed using the primer pairs of EF1728F (5`-CATCGAGAAGTTCGAGAAGG-3`) (Chaverri and Samuels, 2003) and TEF1LLErev (5`-AACTTGCAGGCAATGTGG-3`) (Jaklitsch *et al*., 2005). The PCR cycling conditions consisted of an initial denaturation step of 95°C for 2 min and being subjected to 35 cycles of the following program: 95°C for 45 s, 58°C for 1 min, 72°C for 1 min and final extension at 72°C for 5 min. The PCR products were visualized by ethidium bromide staining on 1.5 % agarose gel electrophoresis.

2.6.4 Sequence analysis and identification of the Trichoderma isolates

The Tef1α gene sequences were subjected to the BLAST search program (National Center for Biotechnology Information) to find a similarity index between sequences. Each sequence was subjected to an individual BLAST search to be verified in GenBank. The BLASTn similarity search program was used to find homologous sequences against the NCBI nucleotide database that confirmed the species-level similarity with the query sequence of the isolates. However, due to commercial interest, the isolates were not submitted to any GenBank yet.

2.7 Screening of Trichoderma strains for their antagonistic properties

2.7.1 Dual-culture assay

The *Trichoderma* isolates were screened for their biological control potential of *B. fabae* using a dual culture assay according to Dennis and Webster (1971). In this method, a 5 mm cut of mycelial agar disc from 5-days' culture of *Trichoderma* isolates were cut and placed on one side of PDA contained in 90 mm diameter petri plate. After 24 hrs. of incubation a 5 mm mycelial agar was cut from the margin of 7 day-old culture of *B. fabae* and placed on the same plate at the opposite side. The plates were incubated at 25±2ºC until the control was fully covered with the growth of *B. fabae*. The antagonistic activity, percent inhibition of radial growth, was calculated according to Morton and Stroube (1955). The radial growth of *B. fabae* against *Trichoderma* isolate was taken using a measuring ruler in millimeters (mm) on the 3rd, 5th, 7th and 9th days of incubation.

$$PIRG = R_1\text{-}R_2/R_1X100\ \%$$

Where, PIRG- percent inhibition of radial growth, R_1- the radial growth of *B. fabae* in the control Petri plate and R_2-the radial growth of *B. fabae* toward the *Trichoderma* isolates in the treated Petri plates. The experiment was conducted three times in triplicates.

The status of antagonism between the *Trichoderma* isolates and *B. fabae* was tested following the method suggested by Bell et al. (1982), with ranking scales of 1-5. Where (1) *Trichoderma* isolates overgrew *B. fabae* and covered above 80 % of the medium, (2) *Trichoderma* isolates overgrew *B. fabae* and covered at least 75 % of the medium, (3) *Trichoderma* isolates and *B. fabae* each colonized one half, 50% of the medium surface, and neither

microorganism appeared to dominate each other, (4) *B. fabae* colonized at least two-thirds of the medium surface and (5) *B. fabae* completely overgrew *Trichoderma* isolates and occupied the entire surface of the medium.

2.7.2 Volatile metabolite production-sealed plate assay

Volatile metabolite production was performed by a sealed plate method as described by Mannaa and Kim (2018). From a 7-days old cultures of *Trichoderma* isolates, a 5 mm-diameter of mycelial cuts were placed on the central part of the PDA medium and incubated. At the same time, the center of another PDA plate of the same size of *B. fabae*. The bottom of both plates was then sealed together with Parafilm tape and incubated at 25±2°C until the *B. fabae* was fully grown on the control PDA plates. The mycelial growth pattern of the test pathogen was measured in mm on the 3rd, 5th, 7th, and 9th days of incubation. All the assays were performed in triplicates three times and the PIMG was calculated using the following formula:

PIMG = D_1-D_2/D_1X100, Where, PIMG- percent inhibition of mycelial growth, D_1-the diameter of *B. fabae* in the control plate and D_2- the diameter of *B. fabae* in the treated plate.

2.7.3 In vivo Inhibition-Detached leaf assay

The effect of five *Trichoderma* isolates on the level of *B. fabae* infection was evaluated using detached leaf assay according to Teshome et al. (2013). The faba bean leaves were washed under tap water, surface sterilized with 1 % sodium hypochlorite for 1 min, washed thoroughly with sterilized water, kept in sterilized glass plates and sprayed on their biaxial surface with a small amount of water. The spore concentrations for *B. fabae* and *Trichoderma* species were adjusted to $4.5x10^5$ and $1.0x10^8$ spore mL^{-1}, respectively, using a haemocytometer. The leaf samples were then treated with 3 drops of spore concentration of *B. fabae* pretreated with *Trichoderma* isolates one day before the inoculation of the test pathogen. The controls were treated with pathogen only. All the assays were undertaken in three replications three times and incubated for five days. The severity of the infection on the leaf samples was rated according to Fotopoulos (2008) using a scale (0-3). Where 0= no lesion, 1= flecked lesion, 2=limited lesion and 3=spreading lesion.

2.7.4 Hyphal coiling/interaction Mycoparasitism study

The *Trichoderma* isolates were tested for their ability to parasitize *B. fabae* using the slide culture technique according to Soliman et al. (2016). A grain size (approx. 2x2 mm) of *Trichoderma* isolates and *B. fabae* was grown on the same glass slide containing the PDA blocks (10x20 mm) side by side and incubated at 25±2°C for 72 hrs. After incubation, the slides were observed and photographed under a microscope (OLYMPUS-BX51, Germany) at 400x to check the growth and the presence of hyphal coiling over *B. fabae* and compared to the control.

2.8 Plant growth-promoting properties of the Trichoderma strains

2.8.1Qualitative and quantitative tricalcium p hosphate (TCP) solubilization

The isolates were tested for their ability to solubilize inorganic phosphate on Pikovskaya's (PVK) agar medium using tricalcium phosphate (TCP) as the sole source of phosphate and the amount of solubilization was determined according to Nautiyal (1999) quantitatively, using National Botanical Research Institute Phosphate (NBRIP) medium. The medium contained; (g/L), glucose (10.0), $Ca_3(PO_4)_2$ (5.0), $MgCl_2.6H_2O$ (5.0), $MgSO_4.7H_2O$ (0.25), KCl (0.2) and $(NH)_2SO_4$ (0.1). For quantification, cultures were taken after 3, 6, and 9 days of incubation and centrifuged (Centrifuge, Wagtech international, United Kingdom) at 10,000 rpm for 25 min. The

pH change of the supernatant was measured using a digital pH Meter (NIG 333, New Delhi, India). The amount of phosphorus was detected using Olsen's Method. One mL of the supernatant was taken and quantified at 880 nm using a spectrophotometer (6405UV/Vis., Jenway, England) and the concentration was calculated from the standard curve constructed from known quantities of potassium di-hydrogen orthophosphate, KH_2PO_4 (µg mL^{-1}).

2.8.2 Qualitative and quantitative assay of indole-3- acetic acid (IAA)

The *Trichoderma* isolates' ability to produce IAA was checked qualitatively as described by Hartmann et al. (1983). Spores ($1x10^6$ spores/mL) were extracted from a 5 mm mycelial cuts of 5-day-grown culture and inoculated in 50 mL potato dextrose broth (PDB) amended with L-tryptophan (1 g L^{-1}) in 250 Erlenmeyer flasks and incubated at 25°C on an orbital shaker (ZJZD-III, Shanghai, China) for 72 hr. Uninoculated flasks were included as controls. The cultures were centrifuged (Wagtech International, United Kingdom) at 3000 rpm for 30 min, from which 2 mL of the supernatants were mixed with two drops of orthophosphoric acid and 4 mL of Salkowaski's reagent (50 mL of 70 % perchloric acid and 1 mL of 0.5 M $FeCl_3$ and 49 mL of sterilized distilled water) and incubated at room temperature in the dark for 30 min. The development of the pink color was visually checked as an indicator of IAA production and quantified at 530 nm. The concentration of IAA produced was calculated in µg mL^{-1} using the standard curve sketched from known concentrations of IAA.

2.8.3 Qualitative assay of ammonia production

The qualitative assay of ammonia production by microbes was done according to Cappuccino and Sherman (1992). The *Trichoderma* isolates were grown in 5 mL peptone water at 28±2°C for 4 days. Following the incubation, 1 mL of Nessler's reagent was added to the cultures to detect the development of a yellow color as an indicator of ammonia production.

2.9 Statistical analysis

The statistical analysis was performed by One-Way ANOVA of SPSS version 24. The comparisons among means were done by Tukey HSD at $\alpha = 0.05$. Values were considered significant at $p<0.05$.

3. Results and Discussion

3.1 Cultural characterization and identification of *Trichoderma* isolates

A total of 19 isolates were isolated and characterized on the basis of their cultural and genetic features. The colonies were whitish on the PDA medium at the beginning on the front side and changed to green as they got older within 3-5 days, small and whitish at 25±2°C incubation for 48-72 hrs. However, following sub-culturing onto PDA, the color was changed to greenish, indicating variability (change in color) as a function of time. The edges of cultures remain white, whereas the reverse side of the plates appears yellowish on PDA, the typical characteristics of *Trichoderma* spp. The data showed that 58, 21 and 21 % of the mycelial color of the isolates on the front side of PDA were whitish green, pale green, and deep green, and the same percentage on the reverse side of the cultures was dull yellowish, pale yellow, and yellow, respectively.

The cultural characteristics, together with translational elongation factor 1-alpha gene (Tef1α) sequence analysis on NCBI, identified the isolates as five of the closest species: *Trichoderma afroharzianum*, *Trichoderma harzianum*, *Trichoderma tomentosum*, *Trichoderma* spp., and *Trichoderma orientale*. The translational elongation factor 1-alpha gene (Tef1α) sequence was used for the identification of *Trichoderma* isolates in this study because this

gene has a unique genetic resolution and has good sequence variation to be used in fungal taxonomy and species differentiation, which is also proven to be useful in studying the *Trichoderma* genera (Zhao *et al*., 2011; Alhawatema *et al*., 2019). The relative abundance of the species showed that 42 % of the isolates were *Trichoderma afroharzianum*, 21 % *Trichoderma tomentosum*, 16 % *Trichoderma harzianum*, 16 % *Trichoderma* sp. LSBA1. and 5 % *Trichoderma orientale* (Table 1).

Table 1: Cultural characterization and molecular identification of *Trichoderma* isolates from the rhizosphere of faba bean collected from Arsi and Bale Zones

Isolates	Cultural characteristics (color)		The nearest species from GenBank (Tef1αg)	Similarity (%)	Accession number of the nearest species	Identified strains
	Front	Reverse				
AAUT21	Whitish green	Dull yellowish	*Trichoderma afroharzianum* LESF553	99.60	KT279014.1	*Trichoderma afroharzianum* AAUT21
AAUT14	Whitish green	Dull yellowish	*Trichoderma harzianum* T2018	98.90	MG735712.1	*Trichoderma harzianum* AAUT14
AAUT3	Pale green	Pale yellow	*Trichoderma tomentosum* MIAE00032	98.40	HM176580.1	*Trichoderma tomentosum* AAUT3
AAUT4	Pale green	Pale yellow	*Trichoderma tomentosum* MIAE00032	98.40	HM176580.1	*Trichoderma tomentosum* AAUT4
AAUT35	Whitish green	Dull yellowish	*Trichoderma afroharzianum* CNFG3201	99.20	MH662568.1	*Trichoderma afroharzianum* AAUT35
AAUT6	Whitish green	Dull yellowish	*Trichoderma harzianum* T2018	98.90	MG735712.1	*Trichoderma harzianum* AAUT6
AAUT8	Whitish green	Dull yellowish	*Trichoderma harzianum* T2018	98.90	MG735712.1	*Trichoderma harzianum* AAUT8
AAUT19	Whitish green	Dull yellowish	*Trichoderma afroharzianum* KUC21406	99.20	MN580168.1	*Trichoderma afroharzianum* AAUT19
AAUT10	Deep green	Yellow	*Trichoderma* sp. LSBA1	98.20	KP743134.1	*Trichoderma* sp. AAUT10
AAUT16	Pale green	Pale yellow	*Trichoderma tomentosum* MIAE00032	98.40	HM176580.1	*Trichoderma tomentosum* AAUT16
AAUT17	Deep green	Yellow	*Trichoderma orientale* PPRI 3894	98.80	EU401579.1	*Trichoderma orientale* AAUT17
AAUT30	Whitish green	Dull yellowish	*Trichoderma afroharzianum* LESF553	99.60	KT279014.1	*Trichoderma afroharzianum* AAUT30
AAUT37	Whitish green	Dull yellowish	*Trichoderma afroharzianum* LESF553	99.60	KT279014.1	*Trichoderma afroharzianum* AAUT37
AAUT38	Whitish green	Dull yellowish	*Trichoderma afroharzianum* LESF553	99.60	KT279014.1	*Trichoderma afroharzianum* AAUT38
AAUT39	Deep green	Yellow	*Trichoderma* sp. LSBA1	98.20	KP743134.1	*Trichoderma* sp. AAUT39
AAUT40	Deep green	Yellow	*Trichoderma* sp. LSBA1	99.20	KP743134.1	*Trichoderma* sp. AAUT40
AAUT44	Pale green	Pale yellow	*Trichoderma tomentosum* MIAE00032	98.40	HM176580.1	*Trichoderma tomentosum* AAUT44
AAUT50	Whitish green	Dull yellowish	*Trichoderma afroharzianum* LESF553	99.60	KT279014.1	*Trichoderma afroharzianum* AAUT50
AAUT45	Whitish green	Dull yellowish	*Trichoderma afroharzianum* LESF553	99.60	KT279014.1	*Trichoderma afroharzianum* AAUT45

Tef1αg- translation elongation factor 1-alpha gene

3.2 The antagonistic properties of the Trichoderma strains against Botrytis fabae Sard

The effectiveness of the *Trichoderma* strains in inhibiting the radial mycelial growth of the test pathogen *B. fabae* varied from 7 % up to 88 % within 3-9 days using dual culture assay. Thus, the inhibitory activity of the strains was within the range of 7-59 % and 53-88 % upon 3 and 9 days of incubation. Among the strains, *T. afroharzianum* AAUT21, and *T. harzianum* AAUT14 were the most effective ones, with 58-88 % PIRG at all incubation, whereas *T. tomentosum* AAUT3, *T. tomentosum* AAUT4, *T. harzianum* AAUT6, *T. harzianum* AAUT8, and *T. afroharzianum* AAUT19 were slow at the beginning but showed the maximum inhibition of 80 % after 9 days of incubation. It is interesting to note that *T. afroharzianum* AAUT45 showed a PIRG of 10 % with 3 days of incubation but increased 8-fold (81 %) after 9 days of incubation. All these strains fell within the best Bell's et al. (1982) scoring scales (Table 2). However, *T. orientale* qualified for the moderate inhibition scoring.

Table 2: The effects of the *Trichoderma* strains on the inhibition of radial growth (%) of *Botrytis fabae* upon 3-9 days of incubation using dual culture assay

		Inhibition of radial growth (%) over control (days)				
Sample	**Strains**	**3rd days**	**5th days**	**7th days**	**9th days**	**Bell's scale**
FRSS-13	*Trichoderma afroharzianum* AAUT21	58^{a}	61^{ab}	68^{ab}	85^{a}	1
FRSS-09	*Trichoderma harzianum* AAUT14	59^{a}	65^{a}	72^{a}	88^{a}	1
FRSS-03	*Trichoderma tomentosum* AAUT3	47^{b}	54^{abc}	59^{abc}	83^{a}	1
FRSS-02	*Trichoderma tomentosum* AAUT4	39^{bc}	46^{bcde}	51^{bcd}	81^{a}	1
FRSS-04	*Trichoderma afroharzianum* AAUT35	35^{cd}	42^{cdefg}	47^{cd}	74^{ab}	2
FRSS-01	*Trichoderma harzianum* AAUT6	43^{bc}	48^{bcd}	53^{abcd}	81^{a}	1
FRSS-15	*Trichoderma harzianum* AAUT8	42^{bc}	44^{bcde}	53^{abcd}	79^{ab}	2
FRSS-07	*Trichoderma afroharzianum* AAUT19	47^{b}	48^{bcd}	54^{abcd}	74^{ab}	2
FRSS-02	*Trichoderma* sp. AAUT10	40^{bc}	45^{bcde}	51^{bcd}	65^{ab}	2
FRSS-06	*Trichoderma tomentosum* AAUT16	10^{gh}	24^{h}	35^{d}	65^{ab}	2
FRSS-08	*Trichoderma orientale* AAUT17	27de	39^{cdefgh}	43^{cd}	53^{b}	3
FRSS-14	*Trichoderma afroharzianum* AAUT30	14^{fgh}	29 ^{efgh}	40^{cd}	68^{ab}	2
FRSS-10	*Trichoderma afroharzianum* AAUT37	14^{fgh}	27^{gh}	43^{cd}	67^{ab}	2
FRSS-05	*Trichoderma afroharzianum* AAUT38	15^{fgh}	24^{h}	42^{cd}	64^{ab}	2
FRSS-16	*Trichoderma* sp. AAUT40	18^{efg}	30^{efgh}	42^{cd}	64^{ab}	2
FRSS-09	*Trichoderma tomentosum* AAUT44	19^{efg}	26^{gh}	42^{cd}	74^{ab}	2
FRSS-12	*Trichoderma afroharzianum* AAUT50	7.0^{h}	28^{gh}	43^{cd}	64^{ab}	2
FRSS-11	*Trichoderma afroharzianum* AAUT45	19^{efg}	36^{defgh}	47^{cd}	81^{a}	1
	CV	0.14	0.18	0.11	0.22	-

FRSS"- stands for faba bean rhizosphere soil samples with corresponding number. Mean values of three replications within the same columns labeled with same letter (s) are not significantly different ($p>0.05$) by Tukey HSD analysis of One-Way ANOVA. Numbers refer to antagonism reactions of *Trichoderma* isolates with *Botrytis fabae* based on the scale of Bell et al. (1982) after 7 days of dual growth. CV- indicates the coefficient of variation among means in the same column.

The strains also showed *in vitro* inhibitory activity of 35-72 % over the control, which was similar to 45-78 % inhibitory activities against *B. fabae* after 7 days of incubation over the control, as reported by Bogumił et al. (2013). On the contrary, Barakat et al. (2014) reported that *Trichoderma* spp., inhibited the mycelial growth

of *B. fabae* by 51-78 % after 6 days of incubation. In Ethiopia, Sahile et al. (2011) showed the most effective *Trichoderma* spp., obtained from faba bean phylloplane inhibited *B. fabae* by 48-98 % within a short incubation time of 3 days. The authors argued that effectiveness is affected by different factors such as the source of the isolates, the difference among the species, the metabolites released by the different strains, and/or the way by which an inhibition was determined and the days of incubation. Based on Bell`s scale, 75 % of the strains covered the medium through over-growing *B. fabae* compared to 21 % of the strains that failed to do so after 7 days of incubation.

The other study was undertaken to evaluate the inhibitory activity level, mycelial growth inhibition (MGI) of *B. fabae* by the selected *Trichoderma* strains using a sealed plate method. The MGI over the control was increased from the 3rd-9th days ranging from 13% to 64 % (Figure 2). The most effective strain was *T. afroharzianum* AAUT19, that showed the highest activity upon the 3rd day of incubation, and *T. harzianum* AAUT14, *T. harzianum* AAUT8, and *T. afroharzianum* AAUT19 showed the best antagonistic activity upon the 6th day of incubation without significant difference among them ($P>0.05$). However, upon the final days of incubation, *T. afroharzianum* AAUT19, *T. harzianum* AAUT8, *T. harzianum* AAUT14, and *T. afroharzianum* AAUT19 revealed the best antagonistic activity against the test pathogen, showing insignificant differences among each other. All the strains reached a maximum inhibition of 50-60 % upon the longest day of incubation, except for *T. tomentosum* AAUT4 and *T. harzianum* AAUT6. It is interesting to note that these strains, together with *T. afroharzianum* AAUT45, were the most effective antagonistic strains in a dual culture (PIRG > 80 %) and yet not effective in this test. On the contrary, *T. afroharzianum* AAUT19 and *T. afroharzianum* AAUT35, which displayed relatively lower activity than the best performers in the dual assay, showed better activity in the MGI assay. The difference between the two assays might be related to the efficiency of volatile metabolites produced by the studied strains. In general, the highest % MGI was shown by *T. harzianum* AAUT14 having no significant variation ($p<0.05$) with *T. harzianum* AAUT8 and *T. afroharzianum* AAUT19, both having 63 % of MGI after 3 (62 %) and 6 (64 %) days after incubation. However, there was variation among the study strains that might be caused by the production of different volatiles at different stages by the *Trichoderma* strains.

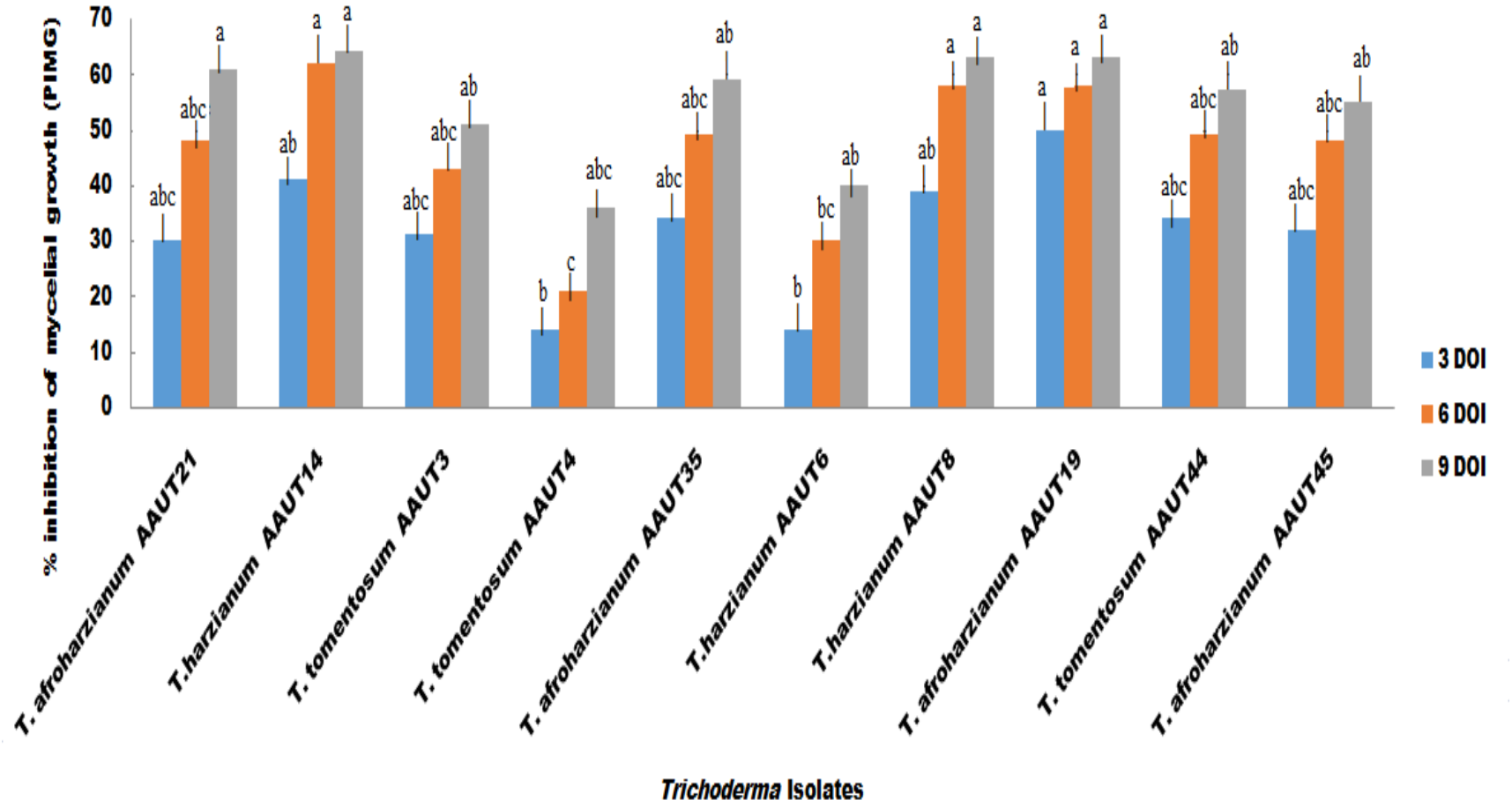


Figure 2. The effects of *Trichoderma* spp. on the mycelial growth of *Botrytis fabae* through sealed plate method. The same letter (s) on the graph represent no significance difference on 3rd, 6th and 9th days of incubation ($p<0.05$) by Tukey`s HSD analysis of One-Way ANOVA

Trichoderma harzianum showed an inhibition of 47 % on *Botrytis* isolates on 7 days of incubation (Bendahmane *et al*., 2012), and the present study also indicated 30-62 % MGI by different *T. harzianum* strains. On the other hand, the *T. harzianum* strains obtained from faba bean leaves exhibited 56-72 % MGI after 7 days of incubation, as reported by Barakat et al. (2014), and Saber et al. (2009) found 50 % MGI of *B. fabae* by *T. harzianum* tag7 on the 6th day of incubation. However, the dual culture assay indicated better inhibition than the sealed plate method to screen *Trichoderma* isolates after 9 days of incubation, whereby a maximum of 64 % and 88 % MGI was displayed by *T. harzianum* AAUT14 on the same days of incubation in the volatile and nonvolatile metabolites assays, respectively. The non-volatile assay conducted by the dual culture method contains the non-diffusible that might be released by the strains into the medium and the involvement of diffusible metabolites that can synergistically contributed for better antagonism than in the sealed plate method (Go *et al*., 2023).

In the detached leaf assay, all the *Trichoderma* strains illustrated the least level of *B. fabae* infection (<2.5 scale) in both faba bean varieties as compared to the respective controls (Table 3). *Trichoderma harzianum* AAUT14, *T. tomentosum* AAUT44, and *T. afroharzianum* AAUT45 allowed the least level of *B. fabae* leaf infection, and the highest leaf infection was seen in the controls. In the Ashebeka variety, treated by *T. harzianum* AAUT14, *T. tomentosum* AAUT44, and *T. afroharzianum* AAUT19, *B. fabae* displayed flecked lesions (1-1.3 mean growth of *B. fabae*). In addition, *B. fabae* showed flecked lesions (0.6 mean growth of *B. fabae*) in the Hachalu variety treated by *T. harzianum* AAUT14 and 1.3 mean growth of *B. fabae* treated by *T. tomentosum* AAUT3 after 5 days of inoculation.

Table 3: The effects of *Trichoderma* strains on the developments of chocolate spot symptoms caused by *Botrytis fabae* on two faba bean varieties (Ashebeka and Hachalu) using detached leaf assay after 5 days of inoculation

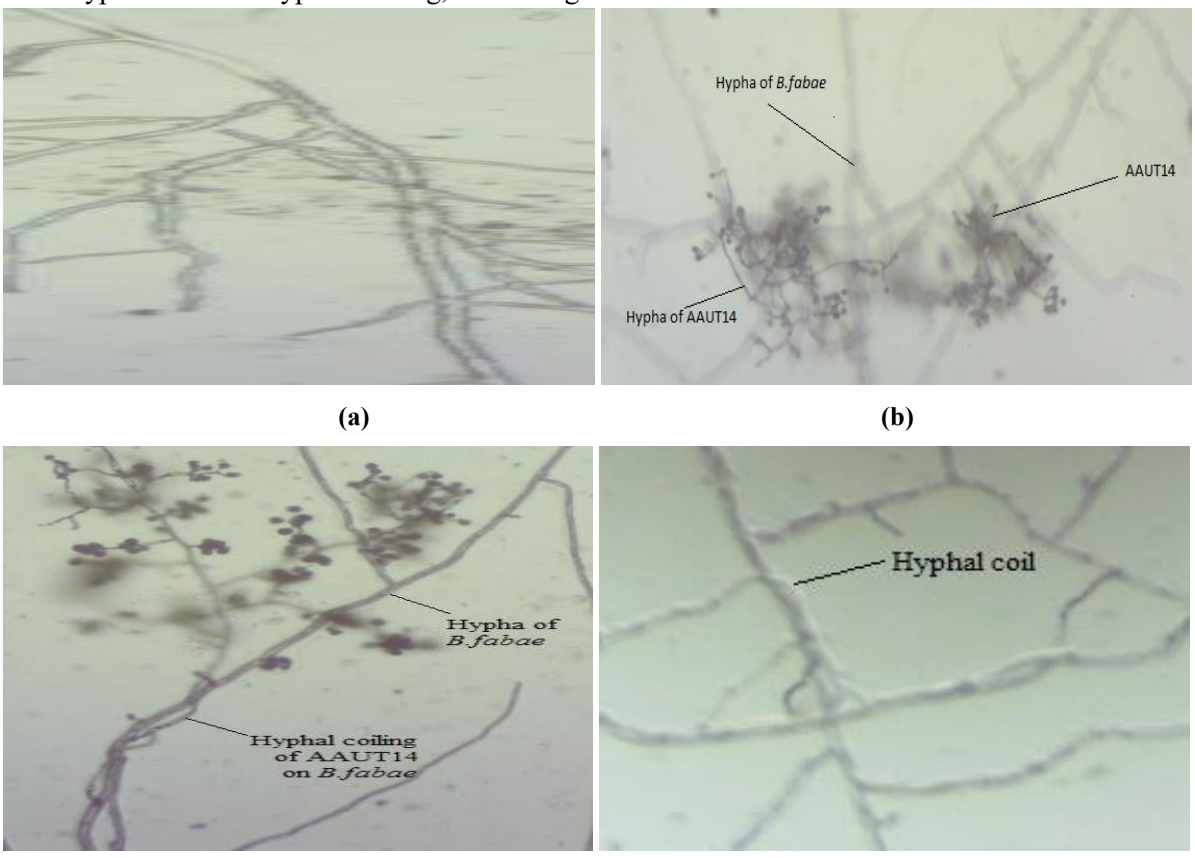
	Mean growth of *Botrytis fabae* (virulence scale 0-3)	
Strains	**Ashebeka**	**Hachalu**
Control	3.00 ^{a}	2.67^{a}
Trichoderma afroharzianum AAUT21	1.67^{ab}	2.00 ^{ab}
Trichoderma harzianum AAUT14	1.00 ^{b}	0.67^{b}
Trichoderma tomentosum AAUT3	2.00^{ab}	1.33 ^{ab}
Trichoderma tomentosum AAUT4	1.67 ^{ab}	1.67^{ab}
Trichoderma afroharzianum AAUT35	2.00 ^{ab}	1.67^{ab}
Trichoderma harzianum AAUT6	1.67 ^{ab}	2.33^{a}
Trichoderma harzianum AAUT8	1.67 ^{ab}	1.67^{ab}
Trichoderma afroharzianum AAUT19	1.33 ^{ab}	2.00 ^{ab}
Trichoderma tomentosum AAUT44	1.00 ^{b}	1.67^{ab}
Trichoderma afroharzianum AAUT45	1.67 ^{ab}	2.00 ^{ab}
CV	0.35	0.36

Mean values in the same column labeled with the same letter (s) as superscript are not significantly different (p>0.05) by Tukey HSD analysis of One-Way ANOVA. CV-indicates the coefficient of variation among means in the same column.

All the strains were significantly different compared to their respective faba bean variety controls (p<0.05) in the protection of *B. fabae* infection and its symptom development on leaf samples of faba beans. This indicates the potential of the strains in prolonging the

incubation period of leaf samples by more than 5 days. The same result was reported by Teshome et al. (2013) using different *Trichoderma* spp. to increase the incubation period of different faba bean leaf samples inoculated by *B. fabae* better than the controls. In addition, the reduced development of chocolate spot symptoms on different faba bean genotypes (Sahile *et al*., 2011).

In the present study, the mycoparasitic properties of all the *Trichoderma* strains on *B. fabae* were also investigated by light microscope using the slide culture method. It was noted that the hyphae of all the *Trichoderma* grew against *B. fabae*, and their hyphae formed hyphal coiling, indicating the mycoparasitic behavior of the strains as represented by *T. harzianum* AAUT14 (Figure 3). Many studies have reported that most isolates of the genus *Trichoderma* act as mycoparasites of many economically important faba bean fungal pathogens. For example, *Trichoderma harzianum* Rifai (Soliman *et al*., 2016) and *Trichoderma reesei* (Magdy *et al*., 2008) mycoparasitized *B. fabae* using slide culture studies. Therefore, in this study, the inhibitory activity of *Trichoderma* spp. in the dual culture method is based on mycoparasitism that can cause hyphal lysis of *B. fabae*.



(a) **(b)**

(c) **(d)**

Figure 3. Mycoparasitism of *T. harzianum* AAUT4 on *Botrytis fabae* (control (a), growth on *Botrytis fabae* hypha (b) and hyphal coiling (c & d))

3.3 Plant growth-promoting properties of the Trichoderma isolates

Trichoderma strains were further characterized for their plant growth-promoting and antagonistic properties under *in vitro* conditions. The data showed that the *Trichoderma* strains that solubilized TCP, produced IAA, and produced ammonia (Table 4). Thus, 47 %, 63 %, and 95 % of the strains solubilized inorganic calcium phosphate, and produced IAA and ammonia, respectively. The strains belonging to *T. harzianum* AAUT14, *T. tomentosum* AAUT4, *T. harzianum* AAUT6, *T. harzianum* AAUT8, *T. orientale* AAUT17, *T. afroharzianum* AAUT37, and *T. afroharzianum* AAUT38 produced the tested plant growth-promoting properties in this study. Indole acetic acid (IAA) production varied considerably among the isolates. The strains produced IAA that ranged from 1.0 to 4.17 µg mL^{-1} indicating that *Trichoderma* spp. obtained from the faba bean rhizosphere can act as a growth promoters of plants through synthesizing beneficial phytohormones. Maximum IAA was produced by *T. harzianum* AAUT6 (4.17 µg mL^{-1}), followed by *T. afroharzianum* AAUT30 (3.34 µg mL^{-1}) and *T. harzianum* AAUT14 (3.16 µg mL^{-1}). The better quantity of IAA was produced by the isolates that showed inhibitory activity against *B. fabae*. Kumar et al. (2017) reported IAA-producing *T. viride* VKF3 exhibited a maximum inhibition of 82 % against *F. oxysporum*.

Table 4: Plant growth-promoting properties of the *Trichoderma* strains from faba bean (*Vicia faba* L.) grown in Arsi and Bale Zones

Strains	IAA (µg mL^{-1})	Ammonia	PS	NPGPP
Control	0.00^{g}	-	-	-
Trichoderma afroharzianum AAUT21	2.36^{cd}	+	-	2
Trichoderma harzianum AAUT14	3.16^{bc}	+	+	3
Trichoderma tomentosum AAUT3	-	+	-	1
Trichoderma tomentosum AAUT4	1.0^{ef}	+	+	3
Trichoderma afroharzianum AAUT35	1.70^{de}	+	-	2
Trichoderma harzianum AAUT6	4.17^{a}	+	+	3
Trichoderma harzianum AAUT8	1.03^{ef}	+	+	3
Trichoderma afroharzianum AAUT19	1.06^{ef}	+	-	2
Trichoderma sp. AAUT10	-	-	-	0
Trichoderma tomentosum AAUT16	-	+	-	1
Trichoderma orientale AAUT17	1.03^{ef}	+	+	3
Trichoderma afroharzianum AAUT30	3.34^{ab}	+	-	2
Trichoderma afroharzianum AAUT37	-	+	+	2
Trichoderma afroharzianum AAUT38	2.67^{bc}	+	+	3
Trichoderma sp. AAUT39	1.21^{e}	+	-	2
Trichoderma sp. AAUT40	-	+	+	2
Trichoderma tomentosum AAUT44	-	+	-	1
Trichoderma afroharzianum AAUT50	-	+	+	2
Trichoderma afroharzianum AAUT45	2.54^{bcd}	+	-	2

Total (%)	63	95	47	95

NPGPP-Number of plant growth-promoting properties (0-3), PS-Phosphate solubilization, IAA- Indole-3-acetic acid

and µg mL^{-1}-microgram per milliliter L-tryptophan-based *Trichoderma* culture. The higher and the lower µg mL^{-1} stands for the maximum and lower value obtained from the spectrophotometer's reading values. Mean values in the same column labeled with the same letter (s) as superscript are not significantly different ($p>0.05$) by Tukey HSD analysis of One-Way ANOVA.

Similarly, 95 % of the strains produced ammonia, indicating that a majority of the strains were producers of ammonia compared to IAA. Prasad et al. (2017) found that 95.5 and 64 % of *Trichoderma* strains obtained from the rhizosphere region of tomato, are the producers of ammonia and IAA, respectively. However, Mohiddin et al. (2017) revealed 65 % of the *Trichoderma* strains obtained from the rhizosphere of chili fields and Kitchen gardens were positive for IAA, which is different from the present study. The difference might be associated with the hosting plants and/or the strains' potentials.

The production of ammonia may have contributed to the antagonistic property displayed by the *Trichoderma* strains against the studied pathogen acting as a volatile metabolite in this study. The production of ammonia by different *T. harzianum* strains has been widely documented as a means to culminate the pathogens as a result of their cellular toxicity (Rawat and Tewari, 2011). Although *Trichoderma* sp. AAUT10 showed various levels of antagonism in the non-volatile (dual culture) assay; the strains failed to produce IAA, ammonia, and solubilize the inorganic phosphate (TCP). This may show its sole antagonistic role in the proximity of the test pathogens through different mechanisms that were not determined in this study, such as the synthesis of lytic enzymes and siderophores. In general, the antagonistic *Trichoderma* strains displayed multiple plant growth-promoting properties.

The phosphate-solubilizing strains were further tested for their ability to solubilize inorganic phosphate, TCP, in a liquid culture medium quantitatively (Table 5). The different *Trichoderma* strains showed variations in the concentration of solubilized phosphate that ranged from 135 to 575 µg mL^{-1} upon 3-6 days of incubation, exhibiting significant differences with the control. Phosphate solubilization increased from 3^{rd}-6^{th} days and reduced after 6 days. *Trichoderma harzianum* AAUT6 released 338 µg mL^{-1} of solubilized phosphate on the 3^{rd} days, and followed by *T. harzianum* AAUT14

(327 µg mL^{-1}) and *T. tomentosum* AAUT4 (326 µg mL^{-1}). The quantity of released phosphate increased from 180 to 575 µg mL^{-1} after the 3^{rd} days and solubilization reduction was observed after the 6 days of incubation in all the strains, though maximum phosphate was released on the 6 days of inoculation by *T. afroharzianum* AAUT38 (575 µg mL^{-1}). Compared to the respective controls, an increase of 2-7, 4-11 and 1.75-6.89-fold was displayed on the 3, 6 and 9 days of inoculation, respectively. This result was much higher than Kapri and Tewari (2010) study that pointed out 392.96 µg mL^{-1} of solubilized phosphate obtained from *Trichoderma* DRT-1 upon 6 days of incubation. However, Bader et al. (2020) reported a maximum of 288.18 µg mL^{-1} solubilized TCP by *Trichoderma* FCCT 363-2 strains on 6 days of inoculation, which is different from the peak concentration noted in the present study (509 µg mL^{-1}) by *T. harzianum* AAUT14 strain. The variation could be due to the physiological variability between the strains.

Table 5: Phosphate solubilization efficiency of the different *Trichoderma* strains from faba bean (*Vicia faba* L.); the amount of phosphorus released (µg mL^{-1}) (NBRIP liquid) (from inorganic calcium phosphate based upon days of incubation (3-9) (for isolates with PVK"+") and change in the initial pH of medium (pH_o=7)

Strains	TCPS 5 g L^{-1} NBRIP (liquid) (µg mL^{-1}) in days (3-9 days)			pH change (3-9 days)		
	3	6	9	3	6	9
Control	45 ^{f}	47 ^{h}	49 ^{f}	6.4 ^{a}	6.6^{a}	6.5 ^{a}
Trichoderma harzianum AAUT14	327 ^{a}	509^{b}	351 ^{c}	5.5 ^{bcd}	4.3^{bc}	5.0^{bcd}
Trichoderma tomentosum AAUT4	326 ^{a}	428 ^{c}	387^{b}	5.8 ^{b}	4.1^{bcd}	5.2^{bc}
Trichoderma harzianum AAUT6	338^{a}	386^{d}	239 ^{d}	5.0^{de}	4.3^{bc}	5.4^{b}
Trichoderma harzianum AAUT8	135^{e}	216 ^{f}	157^{e}	5.6 ^{bc}	4.5^{b}	5.3^{bc}
Trichoderma orientale AAUT17	236^{c}	294 ^{e}	231^{d}	5.1^{cde}	4.3^{bc}	4.7^{cd}
Trichoderma afroharzianum AAUT37	242^{c}	417^{c}	357 ^{c}	5.1^{cde}	4.1^{bcd}	4.5^{c}
Trichoderma afroharzianum AAUT38	285^{b}	575^{a}	417 ^{a}	5.0^{de}	4.5^{b}	4.9^{c}
Trichoderma afroharzianum AAUT50	143^{d}	180^{g}	135^{e}	4.7 ^{e}	4.2^{bcd}	4.5^{c}
CV	0.30	0.32	0.32	0.18	0.22	0.20

TCPS- Tricalcium phosphate solubilization, and NBRIP- National Botanical Research Institute Phosphate. Mean values in the same column labeled with the same letter (s) as superscript are not significantly different ($p>0.05$) by Tukey HSD analysis of One-Way ANOVA. CV-indicates the coefficient of variation among means in the same column.

The data also showed a steady decrease of 1-2.5 pH units from a pH of 6.6-4.1 during 3-6 days of incubation. This may indicate that phosphate solubilization might be enhanced in acidic conditions, as it was observed on the 6th days after inoculation by *T. afroharzianum* AAUT38 (575 µg mL^{-1}, pH=4.5), *T. harzianum* AAUT14 (509 µg mL^{-1}, pH=4.3), and *T. tomentosum* AAUT4 (428 µg mL^{-1}, pH=4.1). Although *T. tomentosum* AAUT4 displayed a pH of 4.1, the released phosphate was the lowest than observed in the two strains (*T. afroharzianum* AAUT38 and *T. harzianum* AAUT14) on the same days, which may indicate the involvement of other byproducts for TCP solubilization. According to Ribas (2016), the potential of TCP solubilization in *Trichoderma* spp. is not only assisted by medium acidification but also by the production of alkaline phosphatases (ALP). Pearson`s analysis indicated no correlation between pH and TCP solubilization (r = -0.612**) (Table 6). Asea et al. (1998) have found a negative correlation between pH and the amount of solubilized phosphorus in the liquid medium of fungi. However, after the 6th day of inoculation, an increase in the pH was observed that might be caused by alkaline substances that are released by the strains into the medium.

Table 6: The Pearson`s correlation analysis between Phosphate solubilization and the change of pH

Correlations			
		phosphate solubilized	pH change
phosphate solubilized	Pearson Correlation	1	-.612**
	Sig. (2-tailed)		.001
	Sum of Squares and Cross-products	500422.000	-1574.900
	Covariance	19247.000	-60.573
	N	27	27
pH. Change	Pearson Correlation	-.612**	1
	Sig. (2-tailed)	.001	
	Sum of Squares and Cross-products	-1574.900	13.250
	Covariance	-60.573	.510
	N	27	27

**. Correlation is significant at the 0.01 level (2-tailed).

4. Conclusion and Recommendation

This study indicated the antagonistic potential of different *Trichoderma* strains on *Botrytis fabae* by the production of plant growth-promoting traits. *Trichoderma harzianum* AAUT14 showed the best antagonistic feature against *B. fabae* along with different plant growth-promoting properties. Thus, further studies should be conducted using another test pathogen, such as *Fusarium* species, to explore the potentials of *Trichoderma* isolates. The volatile and non-volatile compounds of *Trichoderma* isolates should be analyzed by GC-MS technique and quantified.

Ethical approval

Ethical approval was not applicable for this study

Data transparency

The authors would like to confirm that the data supporting the findings of this study are available within the article and further can be obtained from the corresponding author upon request.

Competing interests

Authors have declared no competing interests exist.

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