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RESEARCH ARTICLE

Root rot /wilt incidence of *Phaseolus vulgaris* and antagonistic effect of *Trichoderma.* spp and *Aspergillus terreus* isolated from rhizosphere soil

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Abstract

Eleven isolates of *Fusarium.* sp was isolated from the naturally infected plants of beans by root rot. All isolated pathogenic fungi were morphologically characterised. The highest pathogenicity isolate was isolated named F-5 (qeft-5) with 86% disease incidence percent, all the other isolates varied in disease incidence degree, therefore F-5 isolate was selected for subsequent experiments. F-5 was identified morphologically as *Fusarium oxysporum*. Four isolates of *Trichoderma* were identified as *Trichoderma. harizunum*, *T. viridae*, *T. hamatum and T. asperllum* and one isolate of *Aspergillus. terreus* was isolated from rhizosphere soil a rounded healthy bean plant and examined for its ability to affect the linear growth of *Fusarium o*xysporum in *vitro*. *Trichoderma* species could significantly reduce the growth linear of (F. 5). Inhibition percent of linear growth of *Trichoderma. harzianum* was 66%, *T. viride* 68%, *T. hamatum* 74٪, and *T. asperllum* was 80٪, while, *Aspergillus terreus* was 98%. To our knowledge, this is the first report of reporting a fungal pathogen producing severe root rot and vascular wilt in common beans, as well as the isolation of *A. terreus* from Upper Egypt's rhizosphere soil. It could be concluded that *Aspergillus terreus* isolated from healthy rhizosphere soil around healthy bean roots could suppress *Fusarium oxysporum* the casual agent of bean root rot in Upper-Egypt in vitro experiment.

Keywords: Bean roots; Pathogenic fungi; Phaseolus vulgaris; rhizosphere

1. Introduction

Phaseolus vulgaris, common beans are an essential source of proteins, minerals, and vitamins for many human populations Approximately 27 million tonnes of dry beans are produced worldwide each year (FAOSTAT, 2016). An estimated 6.5 million tonnes of common beans are produced annually in Africa (FAOSTAT, 2017). Egypt produces around 98,132 tonnes of dry common beans annually on 39,665 hectares of harvested land, as reported by the General Authority of Statistics (FAOSTAT, 2017). Fungi are the primary cause of several illnesses that affect common beans, resulting in an 80–100% reduction in yield (FCR, 2017). Most prevalent bean fungal infections, like *Fusarium* spp.-caused root rot, *Sclerotinia sclerotiorum*-caused white mould, *Rhizoctonia solani*-caused damping-off, and *Macrophomina phaseolina*-caused charcoal rot (Singh and Schwartz, 2010). According to Mendes et al. (2013), soil, especially the rhizosphere, is a rich source of microorganisms, with up to 10^{11} microbial cells per gramme. Microorganisms in soil-plant systems perform a variety of roles, from pathogenic to neutral and helpful (Leach, et al., 2017). While both fungi (10^5–10^6 /g soil) and bacteria (10^7–10^9 /g soil) can be found in soil,

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bacteria have been the subject of the most research (Sylvia, 2005). According to Gadd and Geoffrey (2007), fungi are crucial to the health of soil-plant systems because they aid in the vital functions of microorganisms like decomposing organic matter, stabilising soil structure, facilitating biogeochemical transformations, and facilitating the mobility and bioavailability of nutrients. Among the most common and well-researched beneficial soil microorganisms are mycorrhizal and filamentous fungi, which form the unique microhabitat known as the mycorrhizosphere with plant roots. Examples of these fungi include *Aspergillus*, *Penicillium*, *Mucor*, and *Trichoderma*. *Aspergillus* fungi, which include *A. niger* and *A. flavus* are the two most significant species and are typical examples of active soil filamentous fungi (Park et al., 2017). Despite being a saprophytic fungus that can be found in a variety of soils, *Aspergillus terreus* seems to be less well-known and researched (Kamata and Hirota, 1983). An efficient and environmentally friendly way to deal with fungal diseases is through biological control. Numerous antagonistic microbes are active both *in vivo* and *in vitro*. Among the vast investigations, the most well-known are *Trichoderma* spp. (Shabir-U-Rehman et al. 2013), *Aspergillus* species (Suárez-Estrella et al. 2007), and *Penicillium* spp. (De Cal et al. 2009). The most researched biological control agents (BCAs) for root and shoot diseases are *Trichoderma* spp. (Hajieghrari et al. 2008); they are even used in post-harvest (Woo et al. 2014). The primary determinant of soil quality is its biological profile, which has a significant impact on plant growth and tolerance. (Khalil et al. 2015; Eid et al. 2021). *Trichoderma* fungi can be found in high populations in many types of soil habitats. It spreads swiftly, vies for resources and space, and alters the rhizosphere. Along with being a potent biofertilizer, combats plant pathogenic fungus, promotes plant development, and creates plant resistance. [Mukherjee et al. 2012; López-Bucio et al., 2015). Endophytic *Aspergilli* are promising reservoirs for bioactive compounds (Sharaf et al. 2022). Since the extract of endophytic *A. terreus* contains sixteen essential bioactive components, it exhibits intriguing antifungal efficacy against fungal infections. (Hashem et al. 2022). The main aims of the current research are to isolate and characterize the pathogens associated with common bean root rot and wilt symptoms in Qena, Upper Egypt, and assess their pathogenicity under greenhouse conditions and isolated biocontrol agents from non-infected rhizosphere soil and examine their efficacy antagonistic against the pathogen *in vitro*.

Materials and methods

Source of samples.

Plants with natural infections had their symptoms briefly explained and captured on camera, gathered from several locations within the Qena Governorate, carefully plucked and given a thorough wash under running tap water. Rotted roots were chopped into tiny pieces (approximately 2 mm long) after surface sterilised with 5% sodium hypochlorite, repeatedly cleaned with distilled water, and then dried between filter sheets that had been sterilised.

After sterilising, the pieces were placed in a med ium of potato dextrose agar (PDA) supplemente d with penicillin (20 µL/mL), and incubated at 2 5 ± 1o C. The growth of fungal colonies was mo nitored every day.

Causal organisms' isolation, purification and identification.

The hyphal tip isolation approach proposed by Booth (1985) was used to purify the fungal colonies obtained from the incubated diseased roots. The fungal colonies were identified based on their morphological and microscopical characteristics, as described by Booth (1985) and Barnett and Hunter (1972) and preserved on PDA slants and refrigerated at 5 °C for additional research.

Pathogenicity tests.

On plastic pots at the Plant Pathology Department of the Faculty of Agriculture, South Valley University, Qena, Egypt, the pathogenicity tests for the isolated fungi were conducted under greenhouse condition.

Preparation of the fungal inocula.

Each conical flask included 100 g of barely grains and 100 mL of distilled water were autoclaved, and then the fungal isolates were added using 5–10 cm fungal discs that had been cultivated on PDA for four days., the inoculated flasks were incubated at 25 °C with daily shaking for 14 days.

Preparation of soil and pots and Pathogenicity test

To inoculate the soil, 2% of the fungal inocula was mixed with 100 g/5 kg of sterilized soil in each pot, and immediately irrigated. Four sterile seeds of a commercial variety of common beans were planted in each of the inoculated plastic pots, and kept in a greenhouse with regular watering. As the control, sterilised, non-inoculated barely grains were employed. For every treatment, three replicates were carried out. 21 days after inoculation, the degree of root rot disease symptoms was assessed. The seedlings were taken out of the pots, and any extra soil chunks were taken out using a gentle shake and a water bath for the roots. The severity of root rot was visually scored by assessing necrotic lesions on the roots and hypocotyls using a rating scale of 0-5 described according to Filion et al., (2003). The experiment was set up in a fully randomised block design with two trials carried out. Plant samples were collected from various treatments in order to perform re-isolation techniques on PDA.

Isolation, purification and identification of the bioagents fungi:

Ten millilitres of sterilised water were mixed with one gramme of the rhizosphere soil area surrounding healthy bean plant roots, and the mixture was diluted ten times—by 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. PDA was used to cultivate the dilutions (Zhou et al., 2020). Fungal colonies were selected after 72 hours. Single spore isolation was used to purify putative fungal colonies (Dou et al., 2019). The colony identification was carried out by features listed in Siddiquee (2017) and Gams & Bissett (1998).

Evaluation of antifungal assay

All recovery bioagents fungi were tested against the most virulent isolate of pathogenicity tests.

Five millimeter-diameter discs from each biocontrol agent were taken from the periphery of PDA cultures after four days and put on the side of Petri dishes that contained PDA medium, on the other side of Petri plates, the identical disc of *Fusarium* isolate (F5) cultivated in the same way was inoculated. Three replicates were made. Petri dishes with PDA media inoculated with the pathogen only were served as the control treatments.

Inhibition percentage:

Using the following formula (Harlapur et al., 2007), the inhibition percentage was determined by measuring the radial growth of the fungus growing on control and treated plates: $P\% = 100 \times (C - T) / C$

where T is the average radial growth in plates inoculated with bioagents fungi, C is the average radial growth in control plates, and P% is the percentage of pathogen growth suppression.

Statistical analysis: There were three replicates of each treatment in the fully randomised experimental design. Duncan's multiple range tests (Duncan's test $P>0.05$) were used to differentiate the treatment means obtained after the experiment was performed at least twice (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Natural disease symptoms and sampling

The diseased plants developed floppy, greenish-yellow leaves. A cross-section through the stem and base of the infected plant reveals that the brown spots are entire or incomplete rings that reflect the discoloured vascular tissue. Ultimately, the plant turned dark as its leaves dried (Figures. 1&2). (Sartorato and Rava 1994; Salgado et al. 1996) reported that common bean plant infections root caused yellowing and wilt in plants, especially at the blooming and pod-filling stages and the plants may eventually die. Also, the disease can cause an 80% reduction in yield in susceptible common bean cultivars and is fevered by mild temperatures and high soil moisture.

Figure 1. Symptoms of severe common bean root rot /Wilt under natural condition in field

Figure 2. (A, B and C) Samples of diseased common bean plants infected with root rot /Wilt collected from natural field from different area in Qena -governorate -Upper-Egypt.

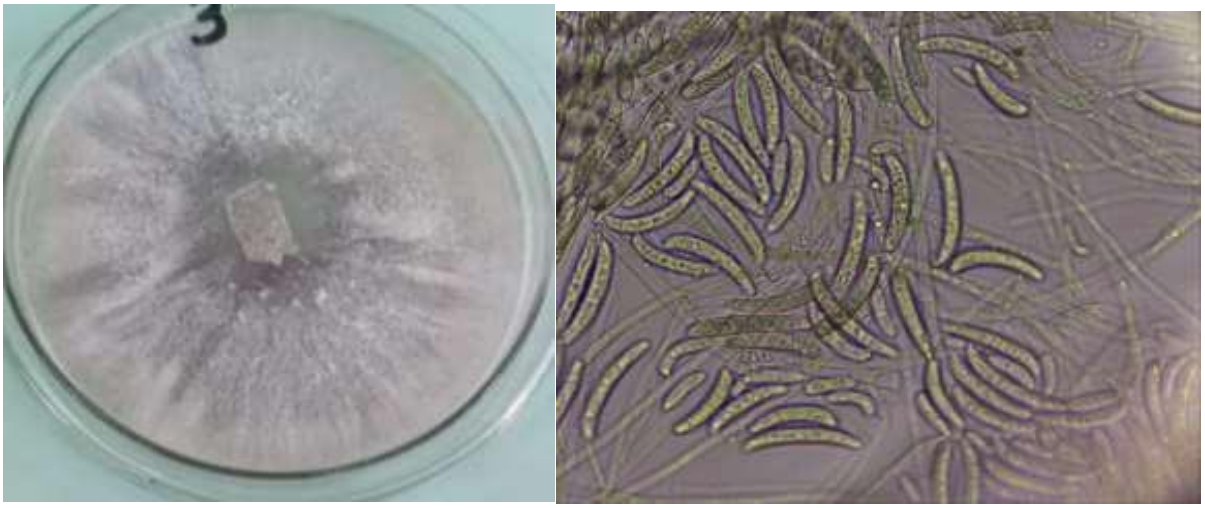

Figure 3. colony of *F*. *oxysporum* grown in PDA medium for five days. Spores of *F*. *oxysporum* under optical microscopy (Macro, micro and chlamidyspores are cleared).

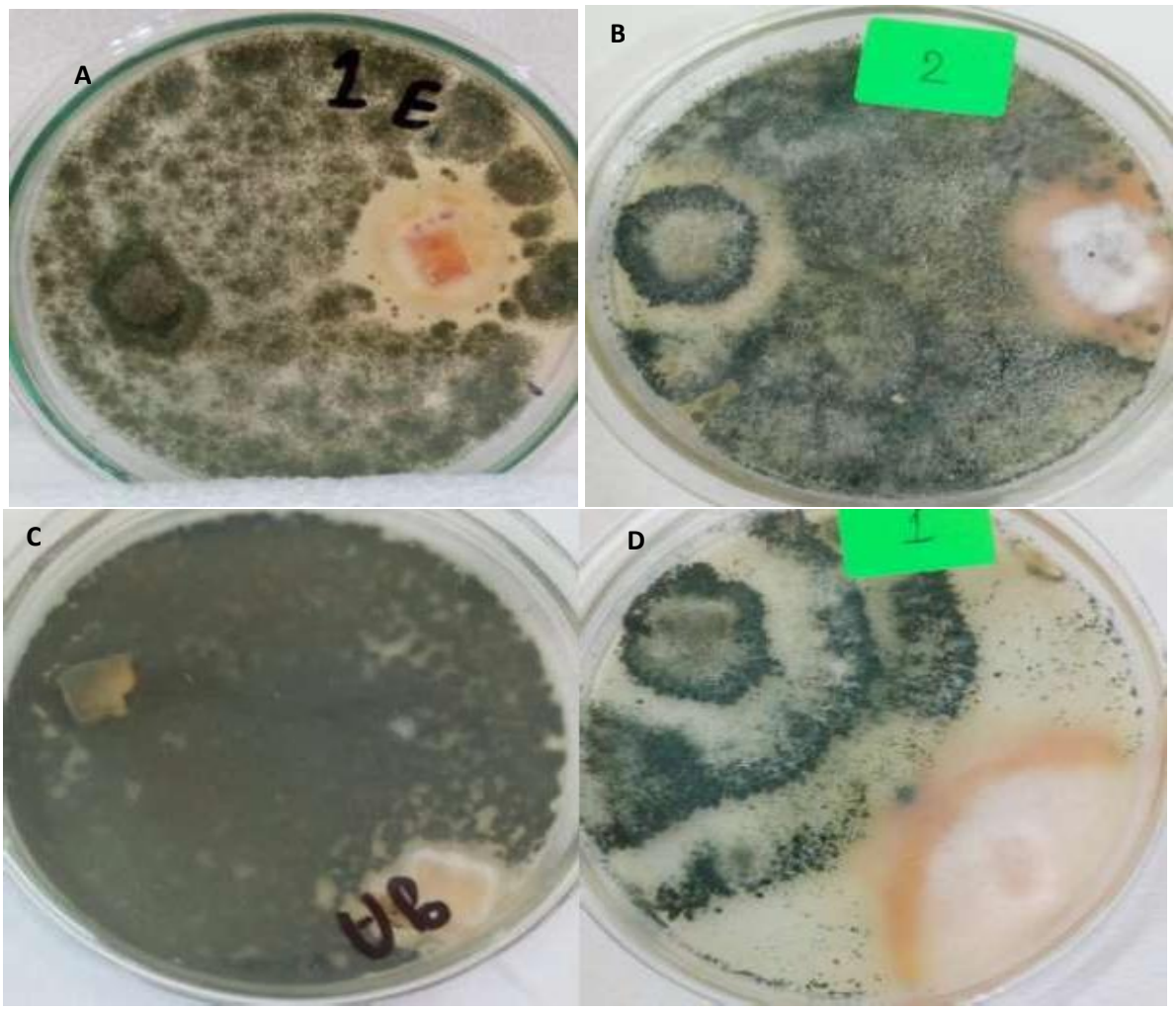

Figure 4. *in vitro* antagonistic with A, (T. viride+F-5), B, (T. harzianum+F-5), C (T. hamatum+F-5) D, (T. asperellum+F-5) growing on perti dishes containing PDAmedium for five days.

Pathogenicity test

The pathogenic fungi could attack common bean plants causing root rot diseases under greenhouse experiment condition. *Fusarium* species was the most frequent of fungal isolates growing from the diseased roots. On growing media, white cottony mycelium with a dark-purple undersurface was seen to assist with the morphological identification of (F5). (Figure. 3). *Fusarium oxysporum* is an asexual fungus that produces three types of spores: microconidia, macroconidia, and chlamydia spores (Figure. 3). According to the pathogenicity test the most severe isolate was *F. oxyrsporum* (F5) isolated from Qeft area in Qena, caused 86 ٪ diseases incidence percent figure (6), however others isolate were able to caused root rot and wilt symptoms with different degree figure (7). Subsequently, isolate (F5) *F. oxysporum* was challenged by antagonistic fungi isolated from healthy rhizosphere soil. *Fusarium oxysporum* f.sp. *phaseoli* (Fop) Kendrick and Snyder is the causative agent of *Fusarium* wilt of common bean, according to results previously confirmed by numerous authors (Kendrick and Snyder, 1942). (Schwartz et al. 2005) reported that the most significant common bean diseases in the world is fusarium wilt caused by (*Fusarium oxysporum f.* sp. *phaseoli* J.B. Kendr. and W.C. Snyder)

Isolation, purification and identification of the bioagents fungi

Four isolates of *Trichoderma.* sp antagonistic fungi were isolated on PDA medium by using dilution plate technique and identified with morphological methods from rhizosphere soil and identified as *Trichoderma. harzianum, T. viride, T. hamatum, T. asperellum.* Bioagents fungi reduced the growth linear of *F. oxysporum* in *vitro* assay (figure.4), the percentage of inhibition growth were as follows, *Trichoderma. harzianum* 66%, *T. viride* 68%, *T. hamatum* 74٪, T. asperllum80٪ and endophytic fungus A. *terreus* was 98% inhibition percent (figure.8). The colonies of *Trichoderma.* sp were initially formed white mycelia then changed into yellowish-green or dark green on the progression of its maturation and spreading. The morphological characteristics of each isolate was determined by the mycelium and conidia formed. There is a significant different between *Trichoderma* isolates effect *in vitro* antagonistic and the most significant one was *T. asperellum* , However, A. *terreus* was most strongest bioagents control than all species of *Trichoderma.* Isolation of *Trichoderm*a spp. from rhizosphere soils was reported by (Elad et al., 1980; Rajkonda and Bhale 2011; Adhikari et al., 2014; Dehariya et al., 2015; Sitansu et al., 2015; Kale et al., 2018). According to Ghanbarzadeh et al. (2014) *Trichoderma* spp. shown antagonism against *F. oxysporum* through the formation of apressoria structures winding around the pathogen. According to Patel and Saraf (2017), *T. asperellum* decreased the incidence of the *F. oxysporum in vitro* and *in vivo* by more than 85%. (Sundaramoorthy and Balabaskar 2013) mention that inoculation of *T. harzianum in vivo* produced the lowest incidence of the disease induced by *F. oxysporum* (15%), whereas *in vitro* inoculation caused radial growth inhibition of *F. oxysporum* by 53% compared to the control. Agriculture has made substantial use of *Trichoderma'*s mycoparasitic biocontrol capabilities since the 1930s, and most study has been done on *T. virens*, *T. atroviride*, *T. hamatum*, *T. asperellum*, and *T. harzianum* (Howell, 2003; Benitez et al., 2004). These processes can be direct mycoparasitism, indirect mycoparasitism through competition for resources and space, antibiosis and stimulation of plant defence mechanisms, or a mix of the two. Through a combination of enzymatic lysis via the secretion of chitinases, glucanases, proteases, and antibiotic synthesis, mycoparasitism entails the direct antagonistic action against soil-borne pathogens (Harman, 2006; Lorito et al., 2015). Colonies morphology used for *A. terreus* identification. Surface colour was gradually altered from light yellow-brownish to rosy brown

on potato dextrose agar, producing small, globose-shaped and smooth-walled conidia that range in colour from light yellow to hyaline, with a diameter of around 2 µm. (Figure. 5).

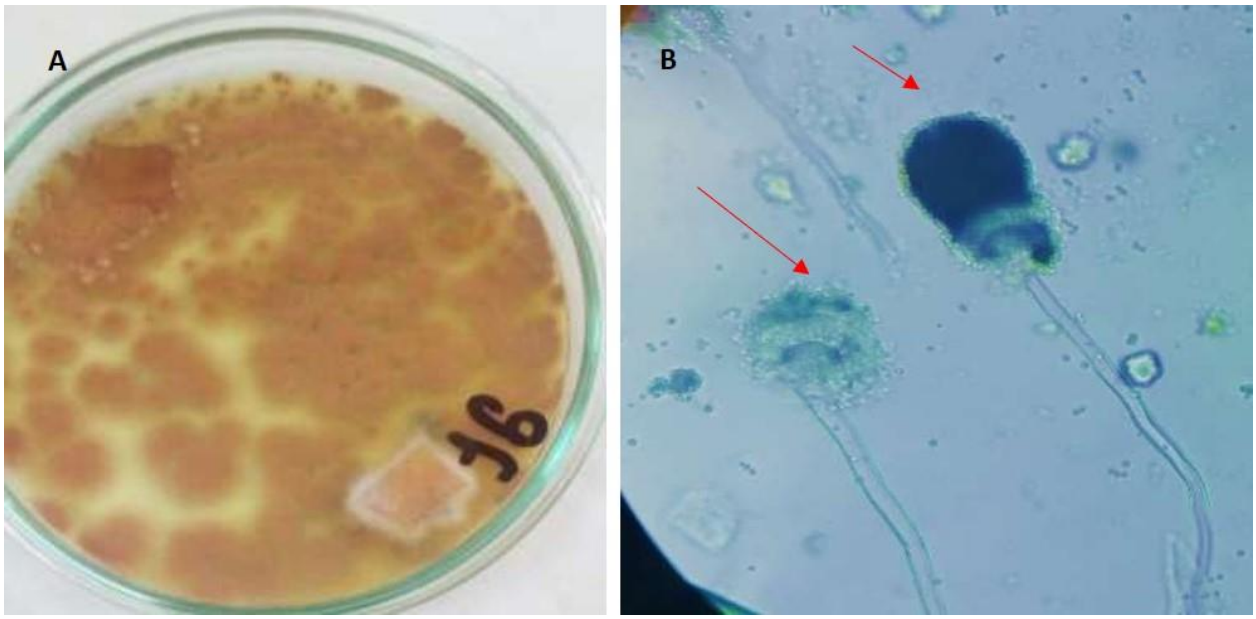

Figure 5.A. in vitro antagonistic with (A . terreus +F-5) on perti dishes containing PDA medium for five days), B. A. terreus spore's morphology under optical microscopy, Conidial heads are compact, columnar and biseriate. Conidiophore stipes are hyaline and smooth-walled. Conidia are globose to ellipsoidal, hyaline to slightly yellow.

Figure 6. Symptoms of common bean plants in pathogenicity test under greenhouse condition, A, inoculated plants with F-5, B, healthy control plants.

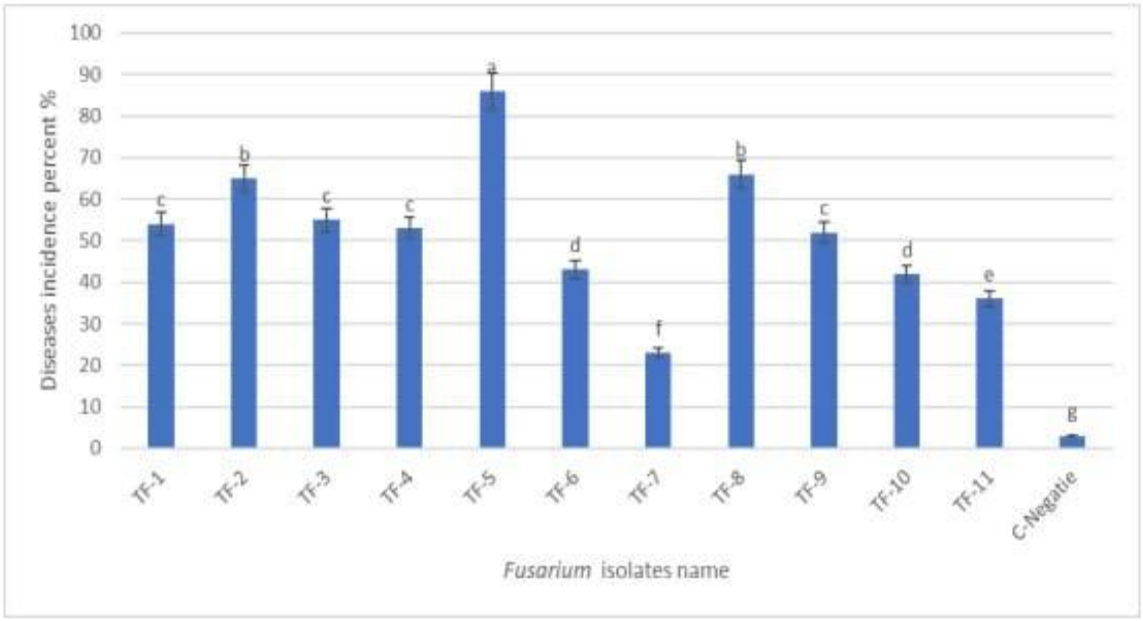

Figure 7. Diseases incidence percent in pathogenicity test under greenhouse condition common bean plants inoculated with eleven isolate of *F. sp.* Values in the column followed by the same letter are not significantly different according to Duncan's at P<0.05.

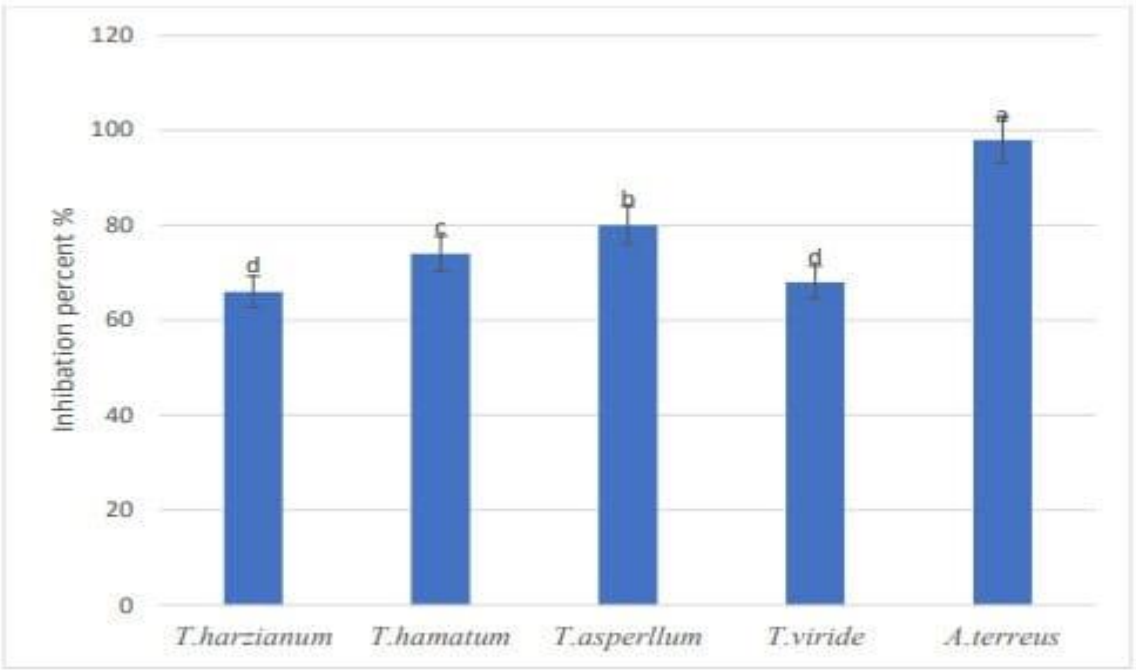

Figure 8. Inhibition percent of growth linear of fusarium isolate (F-5) in vitro antagonis with *Trichoderma* .sp and *Aspergillus.terreus* . Values in the column followed by the same letter are not significantly different according to Duncan's at P<0.05

Colour of spores is one of the most common characters which is widely used in fungal identification and taxonomy, especially among *Aspergillus* and *Penicillium* genera (Sutton et al., 1998; St-Germain and Summerbell, 2003). *Aspergillus terreus* is better known as a biocontrol agent. One significant saprophytic filamentous fungus that is present in soils is *Aspergillus terreus,* extensively researched for its ability to promote plant growth and for its biocontrol properties (Vassileva et al., 2020). Aspergillus play important roles in encouraging plant development and improvement of plant health (Frisvad and Larsen, 2015). According to Ben Abdallah and Khiareddine (2015), several *Aspergillus* species have demonstrated effective antifungal activity against *Phytophthora erythroseptica* and *Fusarium sambucinum* in a dual culture method. Though *A. terreus* is a member of the soil microbiota and is present in a variety of soil types, its ability to enhance plant health and promote plant growth is debatable since it may have either a good or detrimental impact on crops. Thus, more research is needed to determine how *A. terreus* affects various soil types and the mechanism for antagonistic pathogenic fungi (Ben Abdallah and Khiareddine, 2015).

Conclusion

It could be concluded that *F. oxysporum* causes severe root rot in many areas of Qena governorate. *Aspergillus terreus* was the most biocontrol agent isolated from rhizosphere soil around healthy bean plants suppressing 98% of the radial growth of the causal agent of bean root rot *in vitro*.

Authors' Contributions

All authors are contributed in this research

Funding

There is no funding for this research.

Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest.

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