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Mycelial Growth and Identification of Pathogenic Fungus Isolated from Basal Rot Disease of Onion and their Biological Control

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Key words: *Allium cepa*, Basal rot disease, Biological control, *Fusarium verticillioides*, Molecular characterization, Mycelial growth

Abstract

Onions (*Allium cepa* L.) are a major crop for spices in Bangladesh. The current study was conducted to assess the morphological traits, cultural effects, molecular identification, and environmentally friendly management of the pathogenic fungus isolated from onion basal rot disease. *Fusarium verticillioides* causes basal rot symptoms in onion at the early and mature stages. Mycelial growth of *F. verticillioides* was highest on the potato dextrose agar (PDA) medium (72 mm), followed by the potato sucrose agar (PSA) medium, and lowest on the yeast extract agar (YEA) medium (31 mm). *F. verticillioides* grew and developed mycelially best at 30°C and 6.5 pH, respectively. A blast search revealed 99% sequence match with the *F. verticillioides* species complex, and the internal transcribed spacer (ITS) region of the PCR products spanned 590 bp. Against the growth and development of *F. verticillioides* mycelium, *Trichoderma harzianum* exhibited the highest mycelial growth inhibition (75%), followed by *T. erinaceum* (64%), and *T. asperellum* (55%). According to the findings, *T. harzianum*, an antagonistic fungus, is a highly successful bio-control agent against *F. verticillioides*.

Introduction

Onion is widely used as a spice and a highly valued medicinal monocotyledonous plant. It belongs to the Alliaceae family (Musfick et al. 2025). In onion, a modified organ named the bulb is the main edible portion, composed of fleshy leaf sheaths and stem plates (Ali et al. 1998). The inflorescence of an onion is umbel-like and contains 200-600 small individual flowers, which are white or greenish-white in color. Onion seeds are black in color and triangular in cross-section. Onions have three common color varieties, red,

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white, and yellow, with a pungent taste and flavor (Brickell 2022). Diverse health benefits such as anticarcinogenic properties, antithrombotic, antibiotic, antiplatelet and antiasthmatic activity have been reported from the effective bioactive compounds found in onion (Kumar et al. 2015).

Well-drained, fertile soil is best for onion cultivation. At the same time, onion production requires an increasing level of nutrients in the soil. Sandy loams with low sulfur content are good (Boyhan et al. 2001. Furthermore, in the cultivation of onion, short photoperiod and lower temperatures are required for vegetative development of the crop and bulb development. Worldwide production of onion is around 3944 million metric tons per year from 3.17 million hactare. Faridpur, Manikgonj, Dinajpur, Rangpur Rajshahi, Mymensingh, Pabna and Jessore districts are consider as the major onion-growing area in Bangladesh. The total average annual requirement of onion is about 14, 50,000 metric tons, whereas production is 7,25,100 metric tons only, which means 50% of the annual deficit of onion. Low yields are greatly responsible for fungal diseases and a lower quantity of quality onion seeds (Bukhari et al. 2025).

Fusarium species have been reported as plant pathogenic fungi causing various plant diseases on a variety of tropical and sub-tropical plant parts such as roots, fruits, seeds, storage tissues, stems, and vascular wilt. Moreover, isolates of *Fusarium* can spread through soil, and from infected plant debris (Rahman et al. 2024). The maximum mycelial growth, as well as the sporulation of *Alternaria* sp., were recorded at a temperature of 25°C and pH 6.5 on PDA medium (Sultana et al. 2020).

The genomic DNA containing the internal transcribed spacer (ITS) region is highly helpful for identification and evaluating the phylogenetic relationships, as shown by recent molecular phylogenetic research (Alam and Rahman 2020). The ITS region of rDNA is thought to vary between species and even between strains (Alam et al. 2010). There is a prodigious opportunity and need for conducting a comprehensive study on the molecular depiction of *Fusarium verticillioides*, which causes basal rot disease of onion. Therefore, the present research work has been undertaken to isolate and identify the pathogenic fungus that causes basal rot disease of *Allium cepa* using classical fungal taxonomy and molecular techniques. Besides, the effects of cultural conditions and antagonistic fungi on the isolated and identified fungus were also assessed.

Materials and Methods

Basal rot diseased symptoms of onion were collected from commercially cultivated onion fields in Manikganj from October 2024 to January 2025. The collected diseased samples were sealed in sterile polyethylene bags to prevent secondary infection. The laboratory experiments were conducted in the Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. Using the tissue planting methods, a pathogenic fungus was isolated and identified based on the features of the colony, mycelium, conidiophore, and conidia, using standard protocols and related literature (Booth et al. 1971, Akter et al. 2022).

Five different culture media, namely PSA (potato sucrose agar), PDA (potato dextrose agar), GLP (glucose peptone), YEA (yeast extract agar) and HOP (honey peptone), were used in this experiment for the mycelial growth and development of *Fusarium verticillioides*. 15, 20, 25, 30 and 35°C temperatures were tested to find out the optimum temperature for the tested fungus. Five distinct pH levels, *viz.* pH 5.5, 6.0, 6.5, 7.0 and 7.5 were adjusted to the PDA medium using standard methods (Sikder et al. 2024).

Fresh culture mycelia of the selected pathogenic fungus, *Fusarium verticillioides*, 10 days old, were extracted from PDA media for molecular identification. According to White et al. (1990), certain fungal species use the universal primers ITS1F (5´-TCCGTAGGTGAACCTGCGG-3´) and ITS4R (5´-TCCTCCGCTTATTGATATGC-3´) for rDNA amplification by PCR. A Applied Biosystems 2720 Thermal Cycler PCR apparatus was used to carry out the PCR. The best amplification of the desired products is often obtained after 25-30 cycles. We performed 35 cycles in order to enhance the quality of the PCR products. After the purification of the PCR product, samples were transferred to First BASE Laboratories (SdnBhd, Malaysia) for sequencing. MEGA11 and Bioedit were used to verify the DNA sequences.

To further evaluate the retrieved sequence, the NCBI's BLAST software was utilized. The programs Mega Blast and nucleotide BLAST were selected to compare the ITS sequence using their default configurations. The species was identified using the BLAST lowest expected value and the similarity percentage. Once the sequence was analyzed, it was sent to GenBank. The sequence's accession number was ascertained from the submission outcome. analysis of ITS sequences and molecular species authentication. To create the phylogenetic tree, the nucleotide sequences of each fungal isolate were taken from the GenBank. MEGA 11 software was utilized to perform phylogenetic analysis, and the Clustal-W algorithm (Thompson et al. 1994) was employed for sequence alignment. To undertake phylogenetic inference, the Maximum Parsimony (MP) approach was applied with bootstrap values of 1000 replicate runs (Cho et al. 2010, Lee et al. 2010).

Trichoderma harzianum, *T. asperellum* and *T. erinaceum* were used against *F. verticillioides* following standard methods to evaluate the effectiveness of antagonistic fungi. After that, the sample was kept in an incubator for seven days at a temperature of 27 ± 2°C. To make comparisons easier, the control plates with the fungus under test were also cultivated. Three replications of the experiment were carried out. The experimental plates' radial mycelial growth was assessed after incubation. The percentages of the growth inhibition of the tested fungi by *Trichoderma* were calculated according to Alam et al. (2023).

Data on the mycelial growth and inhibition of the isolated fungus by culture media, temperature, pH, and treatment of environmentally friendly control measures were considered normal using one-way ANOVA with Duncan's post-hoc test in SPSS.

Results and Discussion

Early symptoms of infection in mature plants include leaf yellowing, which is subsequently followed by wilting, curling, and decay. Additionally, brown discoloration may spread into the bulb scales, leading to seedling mortality and inhibited growth (Fig. 1). Bulb infection and decay can occur both before and after harvest. Symptomatological observations confirmed typical signs associated with basal rot.

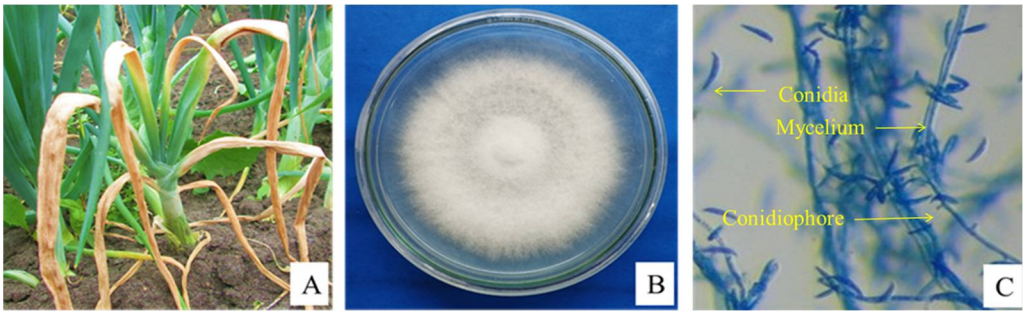

Fig. 1. Identification of pathogenic fungi isolated from basal rot disease of onion through morphological characteristics: (A**)** Symptoms of basal rot disease of onion, (B) *F. verticillioides* colony of seven-day-old culture on PDA medium and (C) Mycelium, conidia and conidiophore of *F. verticillioides*.

These findings are consistent with those described by Cramer (2000), who noted that *F. verticillioides* causes basal rot with these characteristic features under both field and storage conditions. He observed that initial symptoms included yellowing and wilting of leaves, especially the older outer leaves, followed by progressive collapse of the plant from the neck downward. In advanced stages, the basal area of bulbs became brown, necrotic, and shrunken, often showing a white to pinkish mycelial mat on the infected tissue. Again, Leach et al. (2007) found similar findings and according to them, the key features of the basal rot disease observed were the discoloration and rotting of the basal plate and lower bulb scales.

The present study's morphological characterization of *F. verticillioides* revealed distinct features associated with colony development, mycelial growth, and spore formation. These features are critical for the identification and differentiation of *F. verticillioides* from other *Fusarium* species. The mycelium was abundant, aerial and cottony, especially in the early growth phase. The hyphae were hyaline, septate, with smooth margins and branched, as observed under light microscopy. The dense aerial mycelium on PDA provided a favorable environment for sporulation in older cultures. The colony's upper surface was initially white, transitioning to pink as it aged, while the lower surface remained white before developing a pinkish hue upon maturation. The macroconidia were slightly curved and exhibited 3-4 septa. In contrast, the microconidia were single, obovoid, lacked septation and were produced from complex polyphialides

(Fig. 1). The morphological characteristics indicated that the isolated fungus is likely *F. verticillioides*.

According to Ahmmed et al. **(**2022) the presence of profuse, white, and fluffy mycelium is typical of this species, particularly when grown on nutrient-rich media like PDA. Renuka et al. (2023) described *F. verticillioides* as exhibiting a pinkish-white colony color, characterized by hyaline, septate mycelia that displayed verticillate branching, with widths ranging from 2.10 to 5.97 µm. The macroconidia were noted to be elongated with blunt ends (Kaur et al. 2016, Sultana et al. 2022).

Five distinct culture media, potato dextrose agar (PDA), potato sucrose agar (PSA), glucose peptone (GLP), honey peptone (HOP) and yeast extract agar (YEA) were employed to assess the optimal conditions for the growth and development of *F. verticillioides*. The findings indicated that the PDA medium facilitated the most significant mycelial growth, reaching 72 mm, followed by the GLP medium at 56 mm and the PSA medium at 53 mm. Conversely, the YEA medium resulted in the least growth, measuring only 31 mm (Fig. 2). These observations are in agreement with Rahman et al. (2024), who reported that PDA favors vigorous vegetative growth of *Fusarium* species. PDA is a standard culture medium for fungi, which derived from potato infusion and dextrose, provides an ideal carbon source for *F. verticillioides* growth.

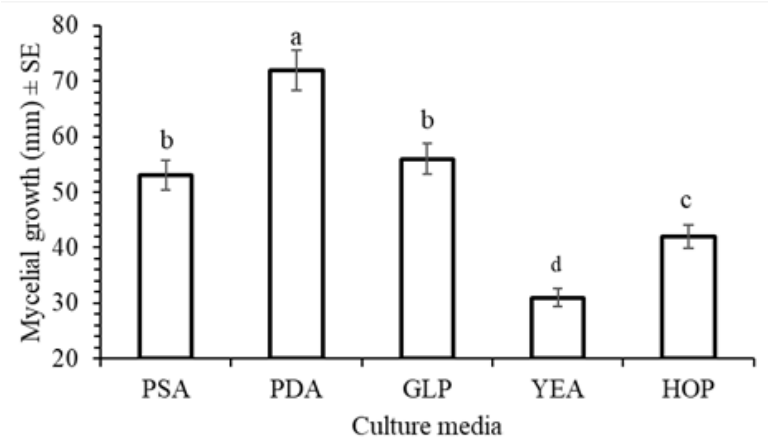

Fig. 2. The effect of culture media on the mycelial growth (mm) of *F. verticillioides* at 7 dpi. Data represents the mean value ± standard error of three replicates. Significant differences are presented as alphabet on the top of the bar and same letter do not differ significantly at 5% level of significant (DMRT).

The present study indicated that the radial mycelial growth of *F. verticillioides* increased steadily until reaching 30°C, after which it began to decline at 35°C. The species achieved its peak growth of 84 mm at 30°C, followed by a growth measurement of 61 mm at 25°C, while the lowest mycelial growth recorded was 24 mm at 15°C (Fig. 3). Poletto et al. (2020) demonstrated that *F. verticillioides* isolates from maize showed

maximum radial growth at 28°C, with a substantial decline in growth at 15°C and 35°C, which was very similar to our findings. Therefore, temperature is a critical environmental factor influencing the mycelial growth, sporulation and mycotoxin production of *F. verticillioides*. The optimal temperature for fumonisin production by *F. verticillioides* has also been reported to be in the range of 25-30°C, under conditions of high humidity (Marín et al. 2004).

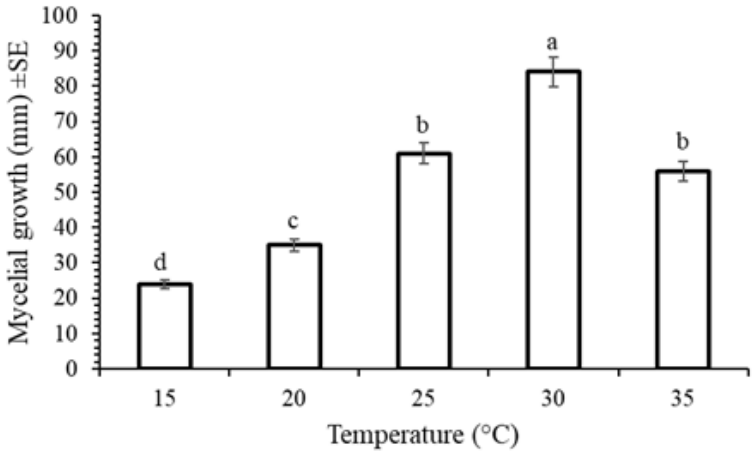

Fig. 3. Mycelial growth of *F. verticillioides* in different temperature regimes at 7 dpi. Data represents the mean value ± standard error of three replicates. Significant differences are presented as alphabet on the top of the bar and same letter do not differ significantly at 5% level of significant (DMRT).

The mycelial growth and development of *F. verticillioides* exhibited an upward trend until reaching a pH of 6.5, beyond which it declined. At this optimal pH of 6.5, *F. verticillioides* demonstrated the most significant mycelial expansion, measuring 72 mm. In contrast, under acidic conditions at pH 5.5, the fungus experienced its least growth, recorded at 35 mm (Fig. 4). In the present study, the fungal isolates exhibited optimal growth under slightly acidic to neutral conditions, particularly within the pH range of 6.0 to 7.0. Colony diameter, pigmentation and sporulation were most prominent at these pH levels. Poletto et al. (2020) found that *F. verticillioides* isolated from maize displayed optimal radial growth at a pH around 6.0, with a notable decline in biomass production at both pH 4.0 and pH 9.0. These results support the conclusion that *F. verticillioides* favors mildly acidic environments, which are often prevalent in natural substrates like decaying plant matter or infected crops. This finding revealed that pH not only influences mycelial growth but also regulates secondary metabolism in *F. verticillioides*.

The molecular identification of *F. verticillioides* in the present study provided a precise and reliable confirmation of the morphological observations. The PCR amplification targeting the ITS region of *F. verticillioides* yielded an amplicon of approximately 590 bp (Fig. 5). Amplification of the ITS region using universal primers

(ITS1 and ITS4) produced a distinct band of approximately 500-600 bp, which is consistent with the expected size range for *Fusarium* species (White et al. 1990).

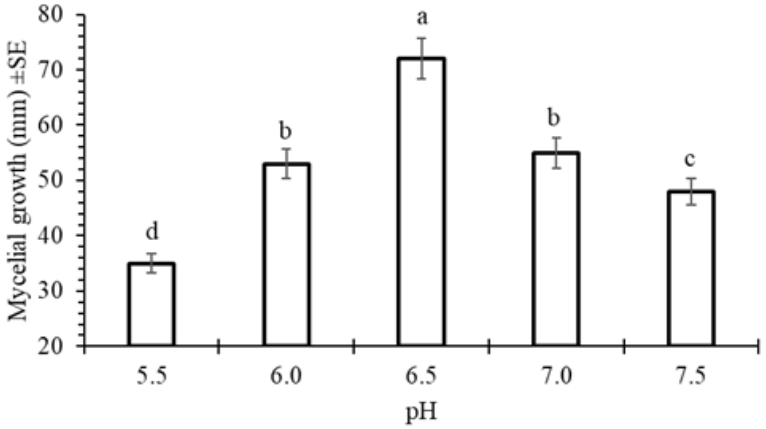

Fig. 4. The effect of pH on mycelial growth (mm) of *F. verticillioides* at 7 dpi. Data represents the mean value ± standard error of three replicates. Significant differences are presented as alphabet on the top of the bar and same letter do not differ significantly at 5% level of significant (DMRT).

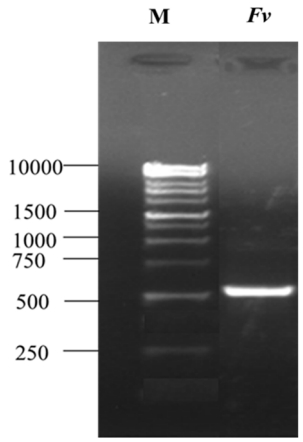

Fig. 5. Profiles of amplification of rDNA-internal transcribed spacer region of the targeted fungi using ITS1 and ITS4 primers. M, molecular size marker (10 kb DNA lader); Lane *Fv* indicates *Fusarium verticillioides*.

The resulting sequence data were then submitted to the National Centre for Biotechnology Information (NCBI) to obtain an accession number. The NCBI processed the ITS sequencing data for *F. verticillioides* and assigned it the accession number PP550014.1 (JUF00084). A BLAST analysis indicated that the organism under investigation, *F. verticillioides* (PP550014.1), exhibited 100% identity with several other sequences of *F. verticillioides*, specifically MN533760.1, MH045733.1, KX783343.1, KP036940.1 and KC752592.1. The nucleotide sequence of the ITS region was utilized to

construct a phylogenetic tree that encompasses 31 representative strains of Nectriaceae, including our taxa *F. verticillioides* (PP550014.1, JUF0084) and an outgroup, *Claviceps purpurea* (FM952769.1). The phylogenetic tree was generated using neighbor-joining (NJ) methods based on the ITS sequence. In this phylogenetic representation (Fig. 6), the taxa are organized into five major clades. Notably, the first and second clades, comprising *F. chlamydosporum* and *F. poae,* clustered together with a bootstrap value of 91%. In the third clade, our examined fungus PP550014.1 clustered with other *F. verticillioides* species, achieving a bootstrap value of 93%. The third, fourth, and fifth clades, which include *F. solani, F. proliferatum and F. oxysporum,* displayed bootstrap values of 92, 92 and 90%, respectively. The close relationship of our studied fungus (PP550014.1) with other *F. verticillioides* taxa supports its classification as a member of the Nectriaceae family.

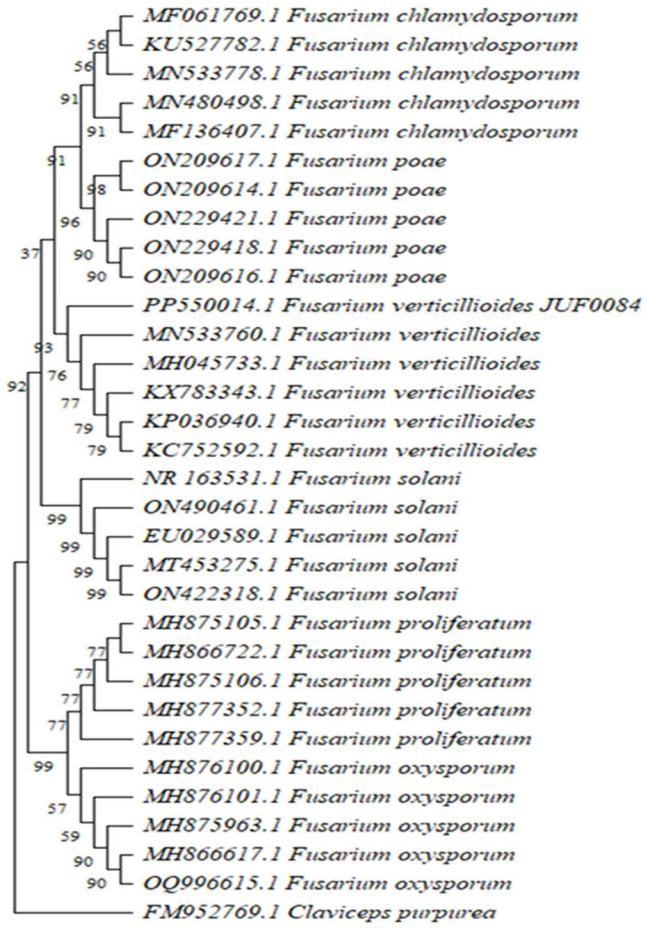

Fig. 6. Phylogenetic tree obtained by analysis of ITS sequence of *F. verticillioides* associated with basal rot disease of onion using neighbor joining (NJ) method with 1000 bootstrapping. *Claviceps purpurea* (FM952769.1) was used as an out-group in the tree.

This high similarity confirms the species-level identity of the pathogen and supports earlier morphological identification. These results align with previous studies where ITS sequencing reliably identified *F. verticillioides* from various hosts, including maize and onion (Abd-Elsalam et al. 2003, Mishra et al. 2020). Again, BLAST analysis of the obtained ITS sequences showed ≥99% similarity to *F. verticillioides* reference strains in the NCBI GenBank database, confirming the identity of the isolates. This aligns with the findings of Poletto et al. (2020), who reported successful identification of *F. verticillioides* isolates from maize based on ITS sequencing, demonstrating its reliability for species-level identification within the genus *Fusarium*.

The result on the efficacy of antagonistic fungi such as *Trichoderma harzianum, T. asperellum* and *T. erenaceum* against *F. verticillioides* is presented in Fig. 7 and 8. The findings indicated that these biocontrol agents significantly reduced the mycelial growth of *F. verticillioides*. The maximum per cent mycelial growth inhibition was observed in *T. harzianum* (75%), followed by *T. erenaceum* (64%) and *T. asperellum* (55%), respectively, against the pathogenic fungus, *F. verticillioides* causes basal rot disease of onion.

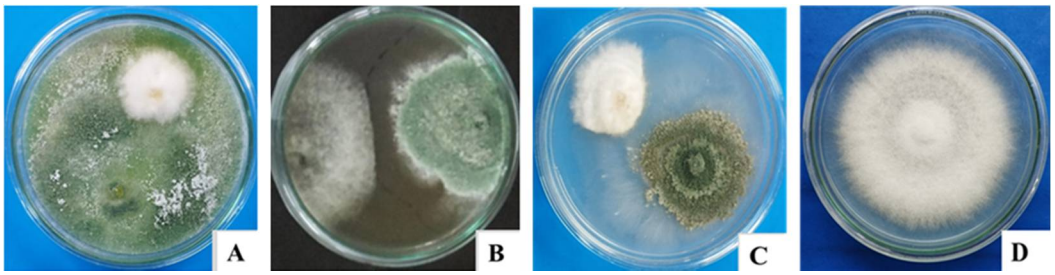

Fig. 7. The mycelial growth inhibition of *F. verticillioides* against selected antagonistic fungi at 7 dpi: (A) *T. harzianum*, (B) *T. asperellum*, (C) *T. erinaceum* and (D) Control.

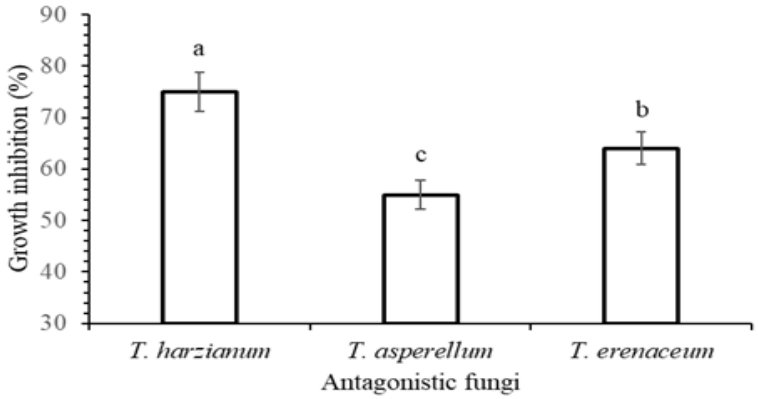

Fig. 8. The mycelial growth inhibition of *F. verticillioides* against selected antagonistic fungi at 7 dpi**.** Data represents the mean value ± standard error of three replicates. Significant differences are presented as alphabet on the top of the bar and same letter do not differ significantly at 5% level of significant (DMRT).

These results are in agreement with earlier studies by Saba et al. (2012) and Singh et al. (2019), who reported similar antagonistic effects of *Trichoderma* spp. on *Fusarium* species. Mycoparasitism was evident in the form of coiling of *Trichoderma* hyphae around *F. verticillioides* mycelium under microscopic observation. Additionally, the presence of inhibition zones in the culture plates indicated the role of secondary metabolites and antibiotics in fungal suppression. This aligns with the findings of Ahmmed et al. (2021), who emphasized the role of lytic enzymes and non-volatile inhibitors produced by *Trichoderma* sp. in suppressing pathogens. Overall, the study confirms that *Trichoderma* spp., particularly *T. harzianum,* is an effective biological control agent against *F. verticillioides*. The findings suggested that *Trichoderma* species could serve as biofungicides against soil-borne plant pathogenic fungi. Nonetheless, additional field research is necessary before the development of biopesticides.

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