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Research Article

Antagonistic Effects of Some Endophytic Fungi against *Colletotrichum coccodes* Causing Anthracnose Disease of Tomato (*Solanum lycopersicum* L.)

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ABSTRACT
Anthracnose fruit rot, caused by *Colletotrichum coccodes*, is a major constraint to tomato (*Solanum lycopersicum*) production worldwide, leading to significant yield and postharvest losses. Conventional chemical control methods pose challenges such as pathogen resistance, environmental contamination, and high costs; hence, the need to explore eco-friendly alternatives. This study investigated the antagonistic potential of endophytic fungi — *Trichoderma* sp., *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus* sp., against *C. coccodes* disease of tomato under *in vitro* conditions. Endophytes were isolated from healthy tomato tissues collected from Adamawa and Gombe States, Nigeria, and identified morphologically. Dual culture assays were used to assess antagonistic interactions. Results showed that all endophytic isolates significantly inhibited the growth of *C. coccodes* ($P < 0.05$), though inhibition levels varied among species. *Trichoderma* sp. exhibited the highest antagonistic activity (up to 12.33 mm inhibition zone at day 7), followed by *Rhizopus* sp. (10.68 mm), *A. niger* (10.52 mm), and *A. flavus* (10.25 mm). Mechanisms observed included antibiosis, mycoparasitism, and competition for nutrients and space. The findings demonstrate that these endophytic fungi possess strong biocontrol potential against tomato anthracnose. *Trichoderma* sp., in particular, could be further developed as a biological control agent within integrated disease management programs. Future studies should focus on field validation and formulation of effective endophytic inoculants for sustainable tomato production.

Keywords: Abattoirs; Epidemiology; Helminths; Livestock markets; Sheep; Zoonosis

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops worldwide, valued for its nutritional and economic importance. However, tomato production is constrained by several fungal diseases, among which anthracnose fruit rot, caused predominantly by *Colletotrichum coccodes*, represents a major yield-limiting factor. The pathogen is both soil- and seed-borne, capable of persisting in infested soils as microsclerotia and surviving on crop debris for extended periods. During warm and humid conditions, conidia are readily dispersed by splashing water, enabling rapid disease spread. Infected fruits initially exhibit small, sunken, circular lesions that expand concentrically and often merge, forming large necrotic areas covered with orange to salmon-coloured spore masses. Severe infections can lead to 30–70% yield losses and a significant reduction in fruit marketability and storag e life (Morsy *et al*., 2019). Conventional control strategies, including sanitation, crop rotation, and chemical fungicides have been used to manage anthracnose. However, excessive reliance on synthetic fungicides has led to high production costs, environmental pollution, and the development of fungicide resistant pathogen strains (Muaz *et al*.,). C onsequently, there is growing interest in exploring eco-friendly and sustainable management approaches (Muaz *et al*., 2024).

Endophytic fungi, which inhabit plant tissues asymptomatically, have emerged as promising biological control agents (BCAs). These microorganisms can inhibit pathogen growth through mechanisms such as mycoparasitism, antibiosis, competition for nutrients and space, and induction of host systemic resistance. Previous studies have reported that endophytic species such as *Trichoderma* sp., *Aspergillus flavus, A. niger*, and *Rhizopus* sp. exhibit notable antagonistic activity against *C. coccodes* (Sopialena *et al*., 2022). The present study, therefore, aimed to isolate and characterize endophytic fungi associated with healthy tomato plants and evaluate their *in vitro* antagonistic potential against *C. coccodes*, with the goal of identifying effective candidates for biocontrol applications.

MATERIALS AND METHODS

Study Area

The study was conducted in two locations namely, Adamawa and Gombe States in Nigeria. Adamawa lies at latitude 9.2092°N and longitude 12.4823°E, while Gombe is situated at latitude 10.2983°N and longitude 11.1773°E. Both areas are within the Northern Guinea Savannah ecological zone, characterized by a tropical continental climate with distinct wet and dry seasons. The rainy season typically begins in late April and ends in October, while the dry season extends from November to March (Adebayo, 2021). Mean annual rainfall averages about 1,200 mm, with temperatures around 29°C. Relative humidity ranges between 60–70% during the wet season and 35–45% during the dry season. The soils in both regions are predominantly sandy loam, suitable for tomato cultivation (Adebayo, 2021).

Isolation and Identification of Endophytic Fungi

Healthy tomato plants and rhizospheric soils were collected from local farms across both study areas. Plant samples were washed, surface sterilized with 0.5% sodium hypochlorite for 30 seconds, rinsed thrice with sterile distilled water, and blotted dry using sterile filter paper. Tissue segments (5 mm) were aseptically plated on Potato Dextrose Agar (PDA) and incubated at 33 ± 2 °C for seven days, following the method of Fajarfika *et al*. (2020). Fungal colonies that grew were sub-cultured repeatedly to obtain pure isolates. The pure cultures were maintained on PDA slants in McCartney bottles and stored at 4 °C for further use. Morphological identification of isolates was based on macroscopic features (colony colour, growth rate, pigmentation, and aerial mycelium) and microscopic observations using lactophenol cotton blue staining. Microscopic structures such as conidia, conidiophores, and sporangiophores were examined under 10x, 40x, and 100x objective lenses of the compound light microscope to confirm fungal identity.

Isolation and Identification of Pathogenic Fungus

Anthracnose-infected tomato fruits were collected and cultured on PDA to isolate the causal pathogen. The colonies were initially greyish-white, turning dark with age and producing black acervuli typical of *Colletotrichum coccodes*. Microscopic examination confirmed the presence of hyaline, single-celled conidia characteristic of the species.

In Vitro Antagonism Test

Antagonistic interactions between the isolated endophytic fungi and *C. coccodes* were evaluated using the dual culture technique described by Fajarfika *et al*. (2020). A 5 mm mycelial disc from each endophytic isolate and the pathogen was placed 3 cm apart on PDA plates. Plates were incubated at 27 ± 2 °C for 7 days. Radial growth of *C. coccodes* was measured at two-day intervals, and the percentage inhibition of mycelial growth was calculated using the following formula:

$l = r1 - r2/r1\ x100\%$, where

L=Pathogenic fungal colonies

r1= Pathogenic colonies away from endophytic fungal colonies (cm)

r2=Pathogenic colonies approaching endophytic fungal colonies(cm)

All treatments were arranged in a completely randomized design with three replicates. Data were subjected to analysis of variance (ANOVA), and means were separated using Tukey's test at (P < 0.05).

RESULTS

Isolation and Identification of Pathogenic Fungus

The pathogen isolated from infected tomato fruits was identified as *Colletotrichum coccodes*. On PDA, the colonies initially appeared greyish-white and later turned dark with age, forming black acervuli at the colony center. Microscopic examination revealed hyaline, single-celled, oval conidia typical of *C. coccodes* (Figure 1). These characteristics confirmed the pathogen responsible for anthracnose fruit rot of tomato.

Frequency of Endophytic Fungi in Tomato Tissues

All four fungal species were detected in different tomato plant parts—roots, stems, leaves, and fruits—indicating their widespread distribution as natural endophytes (Table 1). *Aspergillus niger*, *A. flavus*, and *Rhizopus* sp. each exhibited 75% colonization frequency, while *Trichoderma* sp. recorded 50%, suggesting differential tissue preference.

Isolation and Identification of Endophytic Fungi

our dominant endophytic fungal species were consistently isolated from healthy tomato tissues: *Trichoderma* sp., *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus* sp. (Figures 2–5). Colonies of *Trichoderma* sp. exhibited rapid mycelial growth with greenish pigmentation and irregular margins. *A. niger* and *A. flavus* developed black and yellow-green colonies, respectively, while *Rhizopus* sp. formed white, cottony mycelia. Microscopic observations revealed characteristic conidiophores and sporangiophores confirming their identities.

Antagonistic Activity of Endophytic Fungi against *Colletotrichum coccodes*

Dual culture assays revealed that all tested endophytic fungi significantly inhibited the mycelial growth of *C. coccodes* (P < 0.05), although the degree of inhibition varied among isolates (Table 2). *Trichoderma* sp. exhibited the strongest antagonistic activity throughout the 7-day incubation period, followed by *A. flavus, A. niger*, and *Rhizopus* sp. (Figure 6-9). The control (pathogen alone) showed the fastest mycelial expansion, confirming effective competition and antibiosis by the endophytes.

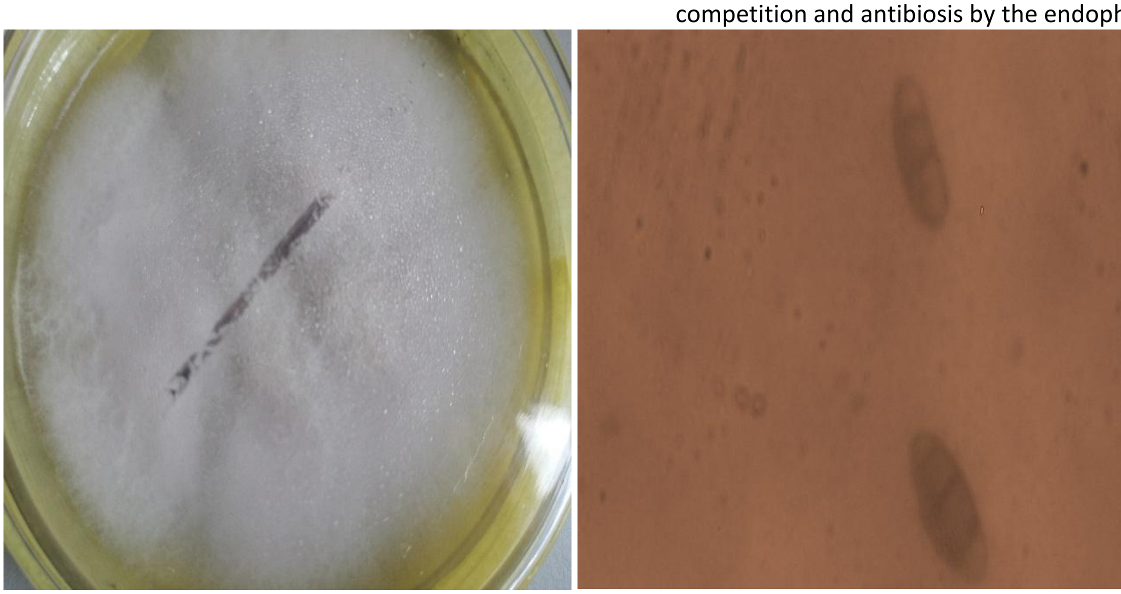

Figure 1. Cultural and morphological characteristics of a seven-day-old *Colletotrichum coccodes* (A: front plate showing mycelial growth B: I and II Conidia at 10x objective, Micro and Macro conidia)

Table 1. Frequency of Endophytic Fungi Isolated from Healthy Tomato Plant Parts

Endophytic isolates	Plant parts				Occurrences	
	Roots	Stems	Leaves	Fruits	Frequency	Percentage
Rhizopus sp.	+	+	+	-	3	75%
Aspergillus niger	+	+	+	-	3	75%
Aspergillus flavus	-	+	+	+	3	75%
Tricoderma sp.	+	+	-	-	2	50%

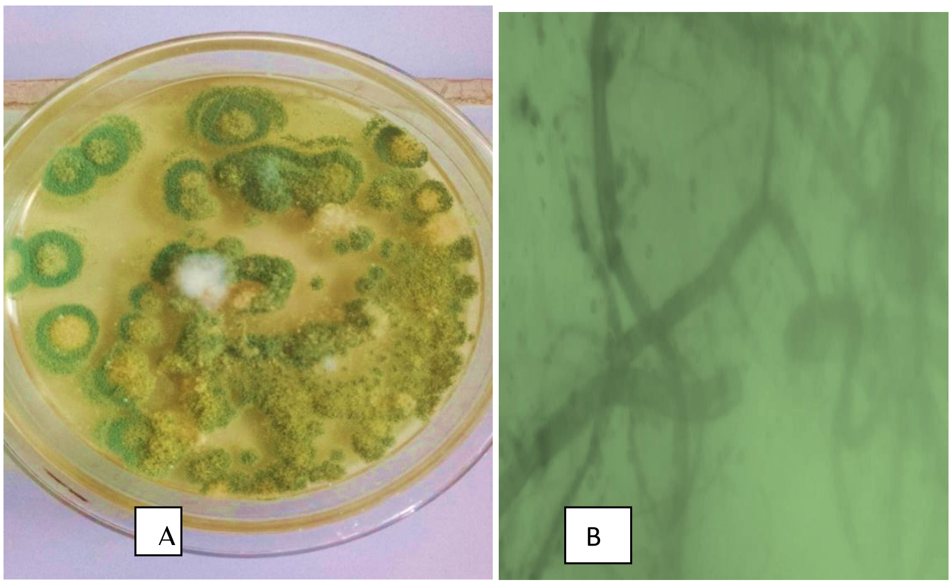

Figure 2. Cultural and Morphorlogical Characteristics of seven days *Tricoderma* sp.
A: front plate showing mycelial growth, B: hypha, *sporangium, sporangiophore* and *sporangiospores* at 10x objective

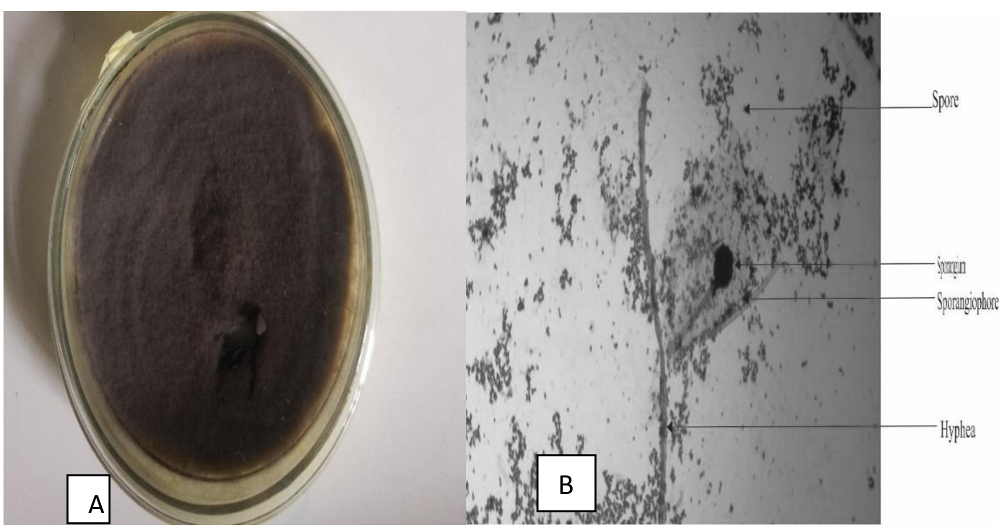

Figure 3. Cultural and Morphorlogical Characteristics of seven days *Aspergillus niger*
A: front plate showing mycelial growth, B: hypha, *sporangium*, *sporangiophore* and *sporangiospores* at 10x objective *Aspergillus niger*

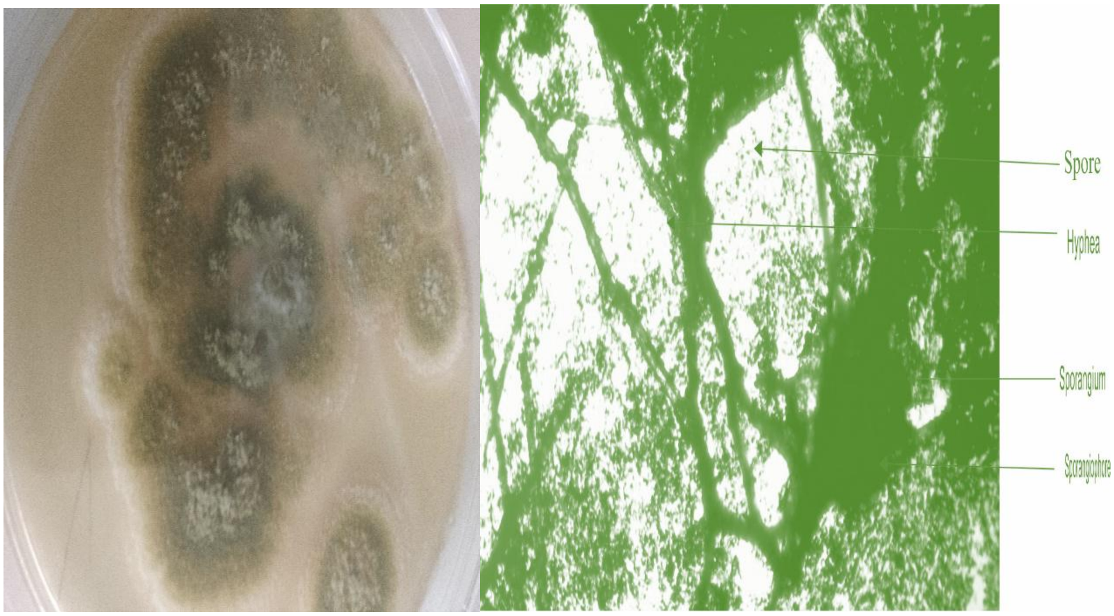

Figure 4. Cultural and Morphorlogical Characteristics of seven days *Aspergillus flavus*
A**:** front plate showing mycelial growth B**:** hypha, sporangium, sporangiophore and sporangiospores at 10x objective

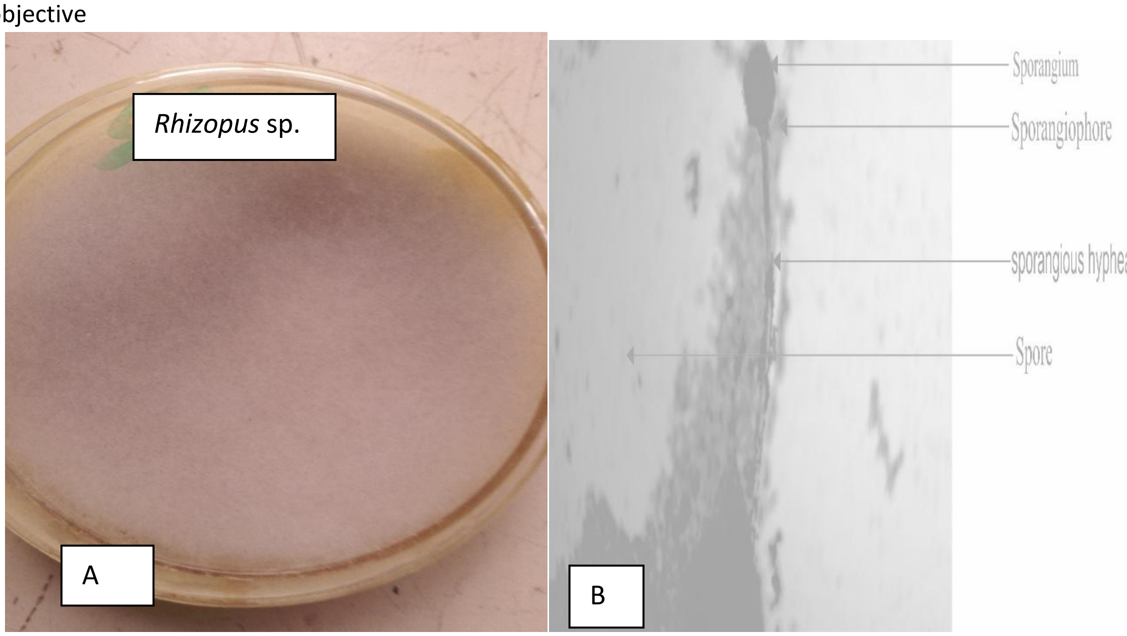

Figure 5. Cultural and Morphological Characteristics of *Rhizopus* sp.
A: front plate showing mycelial growth plate, B: hypha, *sporangium*, *sporangiophore* and *Sporangiospores* at 10x objective

Table 2. ***In-vitro*** **Inhibitory Effect of Endophytic Fungal Isolates Showing Antagonist Activities against Pathogenic Isolate *Colletotrichum coccodes***

Treatments	1	2	3	4	5	6	7
Colletotrichum coccodes	0.6333a	1.5417a	2.6833a	4.0083a	5.475a	6.8667a	8.0583a
Rhizopus+C. coccodes	0.95a	2.6083b	4.6917b	6.6333b	8.0667b	9.4417b	10.6833b
Aspergilus F+ C	1.2583c	3.05b	4.7917b	6.3833b	7.825b	9.075b	10.25b
Aspergilus N+C	1.0833b	2.9583b	4.675b	6.4417b	8.35b	9.425b	10.525b
Tricoderma +C	2d	3.9333c	5.7818b	7.55b	9.1667b	10.5667b	12.3333c

Means followed with the same letters are not Significantly different at ($P < 0.05$) using Tukey test

Keys: *Aspergillus* f.+ *C*= *Aspergillus flavus*+ *Colletotrichum coccodes Aspergillus* n.+ C=*Aspergillus niger+ Colletotrichum coccode*

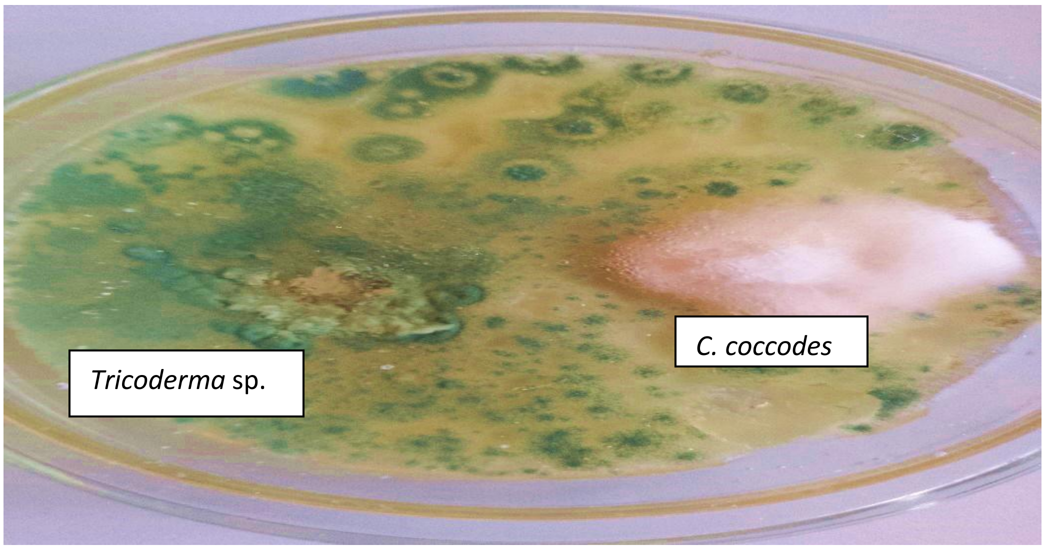

Figure 6. ***Tricoderma*** **sp. Alongside *Colletotrichum coccodes* in dual culture displaying the level of antagonistic activities via antibiosis, mycoparasitism and nutrient competition. At day Seven *in-vitro* trial**

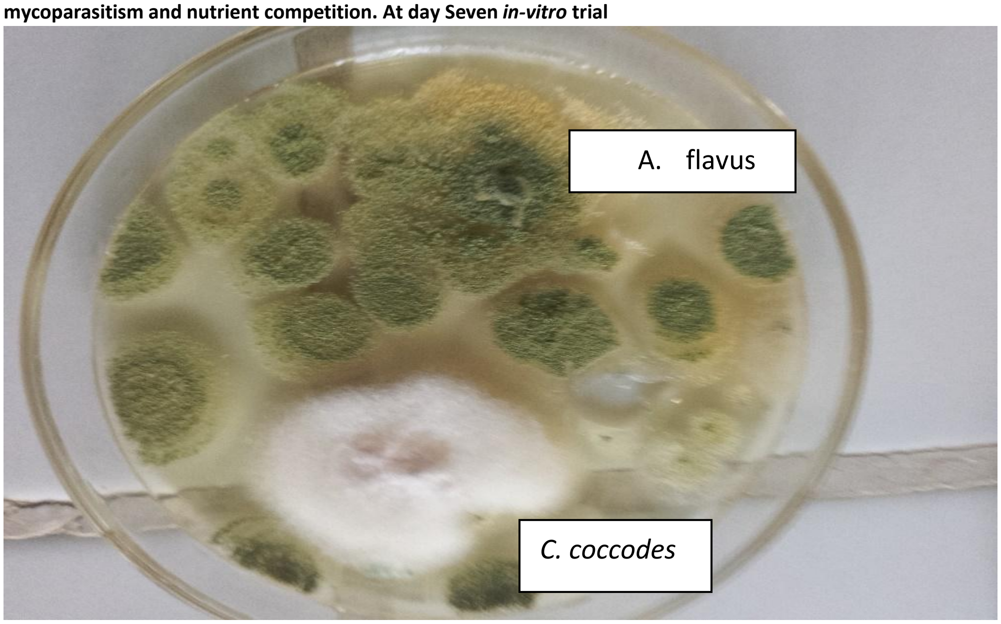

Figure 7. *Aspergillus flavus*. Alongside *Colletotrichum coccodes* in dual culture displaying the level of antagonistic activities via antibiosis, mycoparasitism and nutrient competition. At day Seven *in-vitro* trial

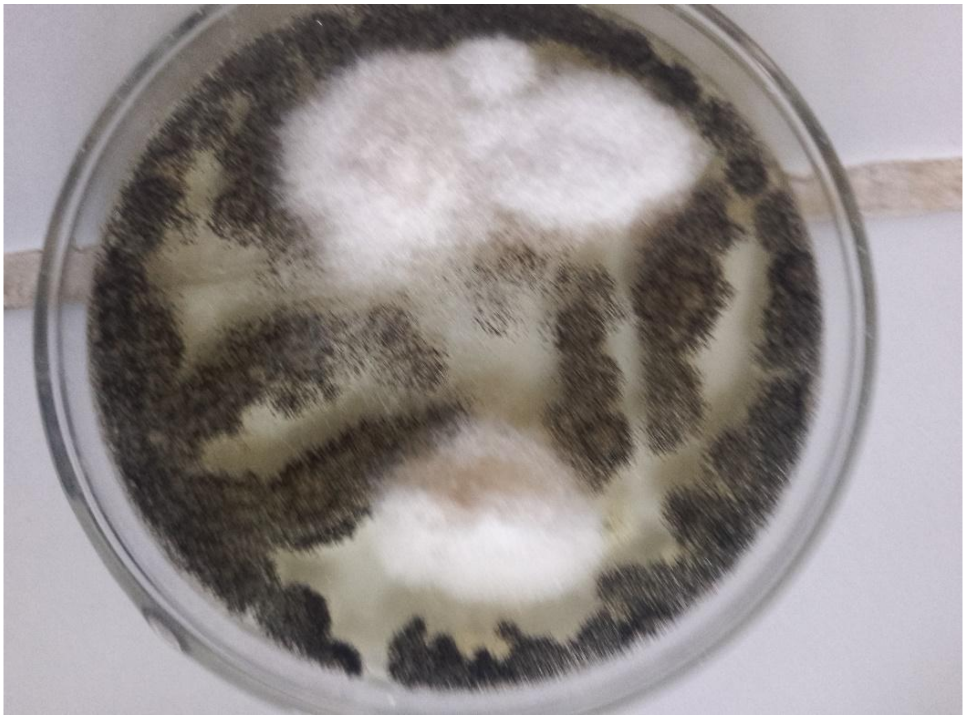

Figure 8. *Aspergillus niger*. Alongside *Colletotrichum coccodes* in dual culture displaying the level of antagonistic activities via antibiosis, mycoparasitism and nutrient competition. At day Seven *in-vitro* trial

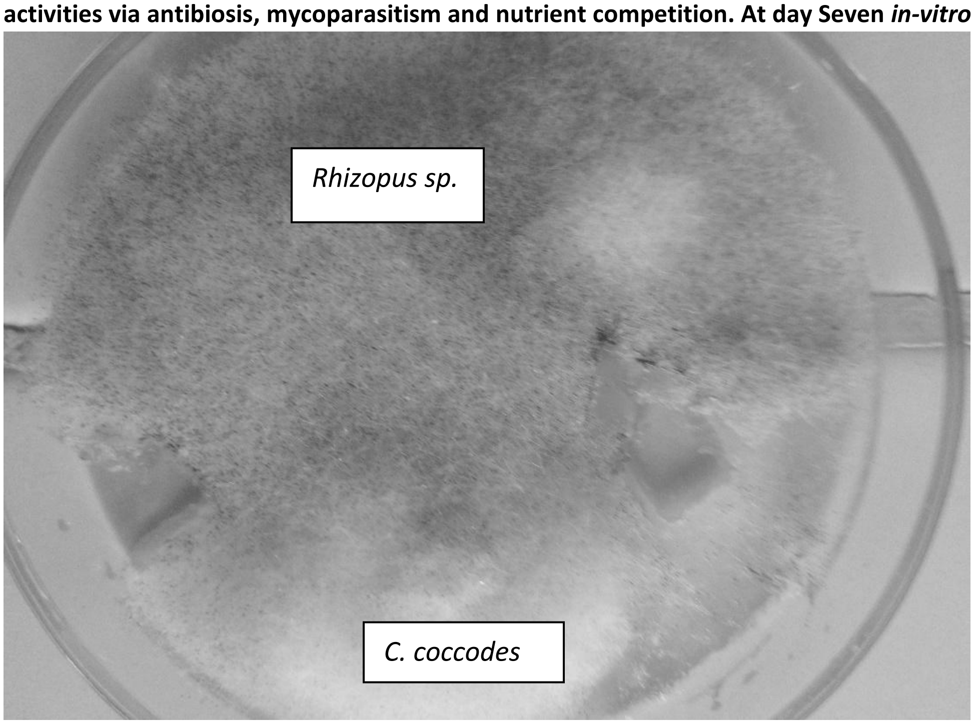

Figure 9. *Rhizopus* sp. Alongside *Colletotrichum coccodes* in dual culture displaying the level of antagonistic activities via antibiosis, mycoparasitism and nutrient competition. At day Seven *in-vitro* trial

Table 2. Inhibitory Effect of Endophytic Fungal Isolates on *Colletotrichum coccodes* in Dual Culture

Treatments	1	2	3	4	5	6	7
Colletotrichum coccodes	0.6333a	1.5417a	2.6833a	4.0083a	5.475a	6.8667a	8.0583a
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Aspergilus F+ C	1.2583c	3.05b	4.7917b	6.3833b	7.825b	9.075b	10.25b
Aspergilus N+C	1.0833b	2.9583b	4.675b	6.4417b	8.35b	9.425b	10.525b
Tricoderma +C	2d	3.9333c	5.7818b	7.55b	9.1667b	10.5667b	12.3333c

Means followed with the same letters are not Significantly different at ($P < 0.05$) using Tukey test

KEY NOTE**;** *Aspergillus* f.+ *C= Aspergillus flavus*+ *Colletotrichum coccodes*

Aspergillus n.+ C=*Aspergillus niger+ Colletotrichum coccodes*

DISCUSSION

The results of this study demonstrate that all four isolated endophytic fungi—*Trichoderma* sp., *Aspergillus flavus, A. niger*, and *Rhizopu*s sp.—exhibited antagonistic activity against *Colletotrichum coccodes*, the causal agent of tomato anthracnose. This finding aligns with previous reports highlighting the ability of endophytes to inhibit pathogenic fungi through diverse mechanisms such as antibiosis, mycoparasitism, and competition for nutrients and space (Triparthi *et al*., 2024). Among the isolates, *Trichoderma* sp. showed the highest inhibitory effect, confirming its reputation as one of the most effective fungal biocontrol agents. The strong antagonistic activity observed in *Trichoderma* species is attributed to their rapid colonization ability, production of lytic enzymes (chitinases, glucanases), and secretion of antifungal metabolites. These mechanisms enable *Trichoderma* to parasitize pathogenic hyphae and disrupt pathogen cell walls (Coppola *et al*., 2019; Patel *et al*., 2021). The significant suppression of *C. coccodes* growth by *Trichoderma* in this study is therefore consistent with its well-documented biocontrol potential in other crops. Although *Aspergillus* species are not typically considered primary biocontrol agents due to potential mycotoxin production, several non-toxigenic strains have been reported to inhibit plant pathogens effectively. The inhibitory effects observed for *A. flavus* and *A. niger* in this research correspond with the results of Sopialena *et al*. (2022), who reported 33–36% growth suppression of *C. coccodes* by similar isolates. These endophytes likely act through antifungal metabolite secretion, competition for essential nutrients, and space exclusion. Additionally, *A. niger* has been shown to produce organic acids and secondary metabolites that inhibit fungal pathogens (Aziz *et al*., 2021). *Rhizopus* sp. also exhibited measurable inhibition of *C. coccodes*, though to a lesser extent. Its suppressive action may result primarily from rapid substrate colonization, thereby restricting nutrient access to the pathogen. This observation agrees with findings from Sopialena *et al*. (2022), who noted that *Rhizopus* isolates exhibited moderate antagonism (~38% inhibition) against *C. coccodes*.

The wide occurrence of these endophytes across all plant parts—roots, stems, leaves, and fruits—suggests they are natural symbionts capable of systemic colonization in tomato. This agrees with reports by Mantzoukas and Eliopoulos (2020) and Patel *et al*. (2021), who noted that *Trichoderma* and *Aspergillus* spp. are capable of inhabiting multiple tissues without causing disease symptoms. However, the detection of *Rhizopus* and *Aspergillus* spp. also warrants caution, as some strains may shift from mutualistic to pathogenic lifestyles under stress conditions (Morsy *et al.,* 2019). Overall, the *in vitro* findings of this study corroborate the hypothesis that endophytic fungi, particularly *Trichoderma* sp., can significantly suppress the growth of *C. coccodes*. The results further strengthen the potential of incorporating endophyte-based bioformulations into integrated disease management (IDM) strategies for sustainable tomato production.

CONCLUSION

Anthracnose caused by *Colletotrichum coccodes* remains a major constraint to tomato (*Solanum lycopersicum*) production, resulting in significant yield and postharvest losses. The findings of this study revealed that tomato-associated endophytic fungi *Trichoderma* sp., *Aspergillus flavus*, *A. niger*, and *Rhizopus* sp. possess distinct antagonistic potentials against *C. coccodes* under *in vitro* conditions. Among the isolates, *Trichoderma* sp. demonstrated the highest level of inhibition, suggesting its strong biocontrol efficacy through mechanisms such as mycoparasitism, antibiosis, and competition for nutrients and space. The ability of these endophytic fungi to colonize tomato tissues asymptomatically and suppress pathogenic fungi highlights their ecological significance and potential as environmentally friendly biocontrol agents. Their integration into tomato production systems could reduce reliance on synthetic fungicides, promoting sustainable disease management practices. Future research should focus on optimizing formulation and delivery methods of these endophytes, as well as validating their effectiveness under greenhouse and field conditions.

Conflict of interest: There was no any conflict of interest between the authors from beginning of the study to the end. Everything was well designed and agreed upon the proposal.

Authors contributions: Conceptualization, P.S.H methodology, P.S.H validation, P.S.H and Z.G.B.; formal analysis, P.S.H and C.I.B.; investigation, P.S.H resources, P.S.H and Z.G.B data curation, P.S.H and C.I.B.; writing original draft preparation, P.S.H.; writing review and editing, P.S.H and Z.G.B.

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