Biological Control of *Fusarium* Wilt in *Corchorus olitorius* Using Indigenous Fungal Antagonists: Integrated In Vitro and Screenhouse Evaluation

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* is a major constraint to the production of *Corchorus olitorius* in tropical agroecosystems, yet information on the effectiveness of fungal biocontrol agents in this crop under West African conditions remains limited. This study evaluated the antagonistic potential of selected indigenous soil fungi (*Trichoderma harzianum*, *Aspergillus niger*, and *Aspergillus flavus*) against *F. oxysporum* using integrated in vitro and screenhouse experiments. Antagonistic activity was assessed using dual culture assays, while a factorial completely randomised design was employed to evaluate disease incidence (DI), disease severity index (DSI), and plant growth responses at inoculum concentrations of 1×10^8 and 1×10^{10} conidia mL^{-1}. All antagonists significantly ($P \leq 0.05$) inhibited pathogen growth in vitro, with *T. harzianum* showing the highest inhibition (68%). Under screenhouse conditions, treatments differed significantly in DI and DSI, with *T. harzianum* at 1×10^{10} conidia mL^{-1} consistently recording the lowest disease levels. Disease severity showed a strong negative correlation with plant height ($r = -0.957$, $P \leq 0.001$), and regression analysis indicated that DSI accounted for 91.6% of the variation in plant height. In addition to disease suppression, *T. harzianum* significantly improved plant growth parameters, including plant height, stem girth, and leaf development, confirming its dual role as a biocontrol and plant growth-promoting agent. *Aspergillus niger* exhibited moderate effects, whereas *A. flavus* showed comparatively lower efficacy. These findings demonstrate the potential of *T. harzianum* for sustainable management strategies for *Fusarium* wilt in *Corchorus olitorius*, although further field validation and molecular characterisation are required for wider application.

Highlights

i. *Trichoderma harzianum* inhibited *Fusarium oxysporium* growth by 68% in vitro.
ii. Disease severity reduced by up to 75% in vivo.
iii. Biocontrol treatments significantly improved plant growth traits.
iv. Antagonistic efficacy varied among fungal species
v. Indigenous fungi show potential for sustainable crop protection.

Introduction

Corchorus olitorius L. (jute mallow) is an important leafy vegetable widely cultivated across tropical and subtropical regions of Africa, Asia, and the Middle East. The crop is valued for its high nutritional content, including vitamins, minerals, and dietary fibre, and plays a significant role in food and nutritional security, particularly among smallholder farming systems in sub-Saharan Africa (Chen et al., 2021; Yao et al., 2023; Awal et al., 2024). Its short growth cycle and adaptability to diverse soil conditions further enhance its importance in peri-urban and intensive vegetable production systems, further enhancing its relevance in sustainable agriculture. Despite its agronomic and nutritional relevance, the productivity of *C. olitorius* is constrained by several soil-borne diseases, among which *Fusarium* wilt, caused by *Fusarium oxysporum*, is one of the most destructive (Ibrahim et al., 2022; Ekwomadu & Mwanza, 2023). The

pathogen is widely distributed in agricultural soils and can infect a broad range of host plants. Infection occurs through the roots, followed by colonisation of the vascular tissues, leading to disruption of water transport, chlorosis, wilting, and eventual plant death. The persistence of *F. oxysporum* in soil through the formation of persistent chlamydospores complicates its management and contributes to recurring disease outbreaks in infested fields (Michielse & Rep, 2009; Dean et al., 2012; Awal et al., 2024). Recent studies have further highlighted the increasing prevalence and economic impact of *Fusarium* wilt in vegetable production systems under intensifying agricultural practices and changing climatic conditions (Ibrahim et al., 2022).

Conventional management strategies for *Fusarium* wilt rely largely on chemical fungicides and soil fumigation. However, these approaches are increasingly limited by environmental concerns, high costs, regulatory restrictions, and the emergence of fungicide-resistant pathogen strains (Ibrahim et al., 2022; Rao et al., 2022; Awal et al., 2024; Rao et al., 2025). Moreover, chemical inputs may adversely affect non-target soil microorganisms and compromise long-term soil health. These limitations have intensified the search for sustainable and environmentally friendly alternatives for managing soil-borne plant diseases (Chen et al., 2021; Harman et al., 2021; Rao et al., 2022; Yao et al., 2023; Jaiswal & Tiwari, 2024; Rao et al., 2025). Biological control using beneficial microorganisms has emerged as a promising strategy for sustainable disease management. Biological biocontrol using beneficial microorganisms has emerged as a promising strategy for sustainable disease management. Microbial antagonists suppress pathogens through multiple mechanisms such as competition for nutrients and ecological niches, production of antifungal metabolites, secretion of lytic enzymes, and induction of systemic resistance in host plants (Yao et al., 2023; Jaiswal & Tiwari, 2024). Among these, fungal antagonists, particularly species of *Trichoderma*, have received considerable attention due to their strong antagonistic capabilities and plant growth-promoting effects (Zin & Badaluddin, 2020; Harman et al., 2021). Recent studies have further demonstrated that *Trichoderma harzianum* can achieve 50–85% suppression of *Fusarium spp.* under controlled and semi-field conditions, while also enhancing plant growth and resilience through modulation of plant defence pathways and rhizosphere interactions (Chen et al., 2021; Mulatu et al., 2023; Wang et al., 2024).

In addition to *Trichoderma*, other soil fungi such as *Aspergillus niger* have been reported to exhibit antagonistic activity against plant pathogens, primarily through organic acid production, nutrient competition, and phosphate solubilization, which may indirectly enhance plant growth (Mundim et al., 2022; Awal et al., 2024). However, the efficacy of *Aspergillus* species as biocontrol agents is often inconsistent and highly dependent on environmental conditions and pathogen characteristics. Furthermore, certain species, such as *Aspergillus flavus*, pose biosafety concerns due to their potential to produce aflatoxins, thereby limiting their suitability for practical field application (Amaike & Keller, 2011; Ibrahim et al., 2022). Although the use of microbial biocontrol agents for managing *Fusarium* wilt has been widely reported in major crops such as tomato, pepper, and legumes, there remains a significant knowledge gap regarding their application in *C. olitorius*, particularly under the edaphic conditions typical of West African agroecosystems. In addition, comparative evaluation of multiple fungal antagonists under integrated experimental frameworks combining in vitro and in vivo (screenhouse) assessments

remains limited. Most existing studies focus on single antagonists or rely exclusively on laboratory assays, thereby providing insufficient insight into their performance under plant-based systems (Yao et al., 2023; Awal et al., 2024).

Furthermore, the effectiveness of biocontrol agents is strongly influenced by soil properties, microbial interactions, and environmental conditions. Sandy loam soils, which are prevalent in many vegetable production systems in West Africa, present unique challenges for microbial establishment and persistence (Ibrahim et al., 2022; Awal et al., 2024; Ávila-Oviedo and Chávez-Avilés, 2026). Therefore, evaluating the performance of indigenous fungal antagonists under such conditions is essential for developing locally adapted and scalable disease management strategies. In this context, the present study aimed to evaluate the antagonistic potential of selected indigenous soil fungi, namely *Trichoderma harzianum, Aspergillus niger*, and *Aspergillus flavus*, against *Fusarium oxysporum* infecting *Corchorus olitorius*. Specifically, the objectives were to: (i) assess the in vitro inhibitory effects of the selected fungi against *F. oxysporum*; (ii) determine their impact on disease incidence and severity under screenhouse conditions; and (iii) evaluate their influence on plant growth parameters.

It was hypothesised that: (i) fungal antagonists would significantly inhibit the growth of *F. oxysporum*; (ii) the degree of inhibition would vary among species due to differences in antagonistic mechanisms; and (iii) effective antagonists, particularly *T. harzianum*, would reduce disease severity and enhance plant growth under controlled conditions.

Materials And Methods

Study area and soil sampling

Soil used for microbial isolation and screenhouse experimentation was collected from the Teaching and Research Farm of the University of Ilorin, Nigeria (8°30'N, 4°35'E), located within the Southern Guinea Savanna agroecological zone. The region is characterised by a unimodal rainfall pattern (1000–1300 mm annually) and mean temperatures ranging from 25 to 32°C. Composite soil samples were collected from the topsoil (0–15 cm depth) using a sterilised auger. Ten subsamples were randomly obtained across the field, homogenised to form a composite sample, and transported in sterile polyethene bags to the laboratory. Samples were air-dried, sieved (2 mm), and stored at 4°C before physicochemical analysis and microbial isolation.

Soil physicochemical characterization

Soil pH was measured in both water and 1 M KCl solutions using a glass electrode pH meter (soil-to-solution ratio of 1:2.5). Organic carbon was determined by the wet oxidation method (Walkley & Black, 1934), and total nitrogen was measured using the Kjeldahl digestion technique (Bremner, 1965). Available phosphorus was extracted using the Bray-I method and measured with a spectrophotometer (Bray & Kurtz, 1945). Exchangeable cations (Ca, Mg, K, Na) were extracted with 1 M ammonium acetate

(pH 7.0) and determined via atomic absorption spectrophotometry (Thomas, 1982). Moist soil sample for microbial analysis was preserved in the refrigerator at 4°C. Soil physicochemical characteristics for both soil conditions are presented in Table I, which establishes a baseline for interpreting microbial interactions and plant growth responses under controlled conditions.

Table 1
Physicochemical properties of sandy loam soil used for the screenhouse experiment

Chemical Characteristics	Soil
pH (H_2O)	7.30
pH (KCl)	6.00
Available Phosphorus (mg P kg^{-1})	0.30
Exchangeable Acidity (cmol kg^{-1})	0.30
Calcium (cmol kg^{-1})	8.15
Potassium (cmol kg^{-1})	2.66
Sodium (cmol kg^{-1})	2.71
Magnesium (cmol kg^{-1})	6.48
Total Exchange Bases	20.00
Organic Carbon (%)	2.52
Total Nitrogen (%)	0.20

Isolation and identification of fungal isolates

Soil fungi were isolated using the serial dilution plate technique on Potato Dextrose Agar (PDA) amended with streptomycin (50 mg L^{-1}) to suppress bacterial growth (Cappuccino & Sherman, 2014; Yao et al., 2023). Plates were incubated at 28 ± 2°C for 5–7 days. Distinct fungal colonies were sub-cultured repeatedly to obtain pure isolates and maintained on PDA slants at 4°C. Preliminary identification was conducted based on macroscopic (colony morphology, pigmentation, growth pattern) and microscopic characteristics (hyphal structure, conidiophores, and spore morphology) using lactophenol cotton blue staining and standard taxonomic keys.

To ensure taxonomic precision and reproducibility, molecular identification using the internal transcribed spacer (ITS) region sequencing is recommended for future validation of isolates.

Pathogen pathogenicity confirmation

Pathogen pathogenicity confirmation
The pathogenicity of *Fusarium oxysporum* was verified using Koch's postulates. A conidial suspension (1 × 10^6 conidia mL^{-1}) was prepared and inoculated into the rhizosphere of healthy *Corchorus olitorius* seedlings grown in sterilised soil. Plants were monitored for symptom development (chlorosis and wilting) over 14 days. The pathogen was subsequently re-isolated and morphologically confirmed, thereby establishing causality.

In vitro antagonistic assay

The antagonistic potential of selected fungal isolates (*Trichoderma harzianum, Aspergillus niger*, and *Aspergillus flavus*) against *F. oxysporum* was evaluated using the dual culture technique (Afifi et al., 2019; Yao et al., 2023; Jaiswal & Tiwari, 2024) (Plates I-II; Figure II). A 5 mm diameter mycelial disc from a 7-day-old culture of *F. oxysporum* was placed at one edge of a 90 mm PDA plate, while a similar disc of the antagonist was placed at the opposite edge. Control plates contained only the pathogen. Plates were incubated at 28°C for 5 days, and radial growth of the pathogen was measured along two perpendicular axes. Each treatment was replicated three times.

Radial growth of the pathogen was measured along two perpendicular axes, and the percentage inhibition of radial growth (PIRG) (Vincent, 1947; Awal et al., 2024) was calculated as:

$$PIRG = \frac{R_1 - R_2}{R_1} \times 100$$

where:

R_1 = radial growth control plates (mm)

R_2 = radial growth in dual culture plates (mm)

Each treatment was replicated three times.

Preparation of fungal inoculum

Fungal inocula were prepared from 7-day-old cultures grown on PDA. Conidia were harvested by flooding plates with sterile distilled water and gently scraping the surface. The suspension was filtered through sterile muslin cloth to remove mycelial fragments. Spore concentrations were determined using a haemocytometer and adjusted to 1 × 10^8 and 1 × 10^{10} conidia mL^{-1}. representing moderate and high inoculum densities commonly used in biocontrol efficacy studies.

Screenhouse experimental design

A screenhouse experiment was conducted using a completely randomised design (CRD) (Gomez & Gomez, 1984). Although the treatments were conceptually derived from combinations of pathogen inoculum levels and antagonist types, they were implemented and analysed as nine discrete treatment combinations, each representing a biologically relevant interaction scenario between pathogen and antagonist.

The treatments included:

1. Control (no pathogen, no antagonist)
2. *Fusarium oxysporum* only (10^8 conidia mL^{-1})
3. *Fusarium oxysporum* only (10^{10} conidia mL^{-1})
4. *F. oxysporum* + *Trichoderma harzianum* (10^8 conidia mL^{-1})
5. *F. oxysporum* + *Trichoderma harzianum* (10^{10} conidia mL^{-1})
6. *F. oxysporum* + *Aspergillus niger* (10^8 conidia mL^{-1})
7. *F. oxysporum* + *Aspergillus niger* (10^{10} conidia mL^{-1})
8. *F. oxysporum* + *Aspergillus flavus* (10^8 conidia mL^{-1})
9. *F. oxysporum* + *Aspergillus flavus* (10^{10} conidia mL^{-1})

Sterilised sandy loam soil (autoclaved at 121°C for 60 minutes on two consecutive days) was dispensed into 5 kg plastic pots. Each pot was considered an experimental unit and was maintained under ambient screenhouse conditions (temperature 26–32°C; natural photoperiod). Each treatment was replicated three times, giving a total of 27 pots. Although soil sterilisation ensured controlled pathogen-antagonist interactions, it may reduce ecological realism by eliminating native microbiota. This limitation is addressed in the Discussion.

Planting and inoculation procedure

Seeds of *Corchorus olitorius* were surface sterilised using 70% ethanol (30 s) and rinsed thoroughly with sterile distilled water. Seeds were sown directly into pots and thinned to two uniform seedlings per pot after emergence. Pathogen inoculation was carried out at 7 days after emergence using a soil drench method (10 mL suspension per pot). Antagonistic fungi were applied simultaneously to ensure competitive establishment in the rhizosphere.

Measurement of plant growth parameters

Growth parameters were measured at 2, 3, 4, and 5 weeks after planting (WAP). The parameters included: plant height (cm), stem girth (mm) using a digital calliper, number of leaves per plant, leaf length (cm), and leaf width (cm). At harvest, plants were carefully uprooted and washed for accurate measurement.

Disease incidence and severity assessment

Diseases incidence (DI) was calculated as:

$$DI\ (\%) = \frac{Number\ of\ infected}{Total\ number\ of\ plants} \times\ 100$$

Disease severity of *Fusarium wilt* on *Corchorus olitorius* was evaluated using a 0–5 ordinal scale based on visual symptoms:

0 = No visible symptoms

1 = Slight yellowing

2 = Moderate yellowing, slight wilting

3 = Severe wilting, leaf drop

4 = Extensive wilting, vascular browning

5 = Plant death

Disease severity index was calculated for each treatment using the formula:

$$DSI\ (\%) = \frac{\sum\ (n_i \times\ S_i)}{N\ \times\ S} \times\ 100$$

where,

n_i = number of plants in each severity class

S_i = severity score

N = total number of plants assessed

S = maximum disease (5)

Assessments were conducted at 2,3,4, and 5 WAP across all treatments.

Statistical analysis

All data were analysed using GENSTAT (version 12, VSN International, UK). The experiment was analysed as a one-factor completely randomised design (CRD), where nine treatment combinations were considered as a single factor. This approach was adopted to enable direct comparison of treatment-specific biological responses, which represent integrated effects of pathogen pressure and antagonist activity. Although the experimental setup involved multiple components (pathogen level and antagonist type), the primary objective of the study was to evaluate the net effect of each treatment combination

rather than to partition variance into interaction effects. Consequently, treatment-based analysis of variance (ANOVA) was considered appropriate and consistent with similar biocontrol studies.

Percentage data (DI and DSI) were arcsine square-root transformed before analysis to stabilise variance. Treatment means were separated using the Least Significant Difference (LSD) test at $P \leq 0.05$. To quantify the relationship between disease severity and plant growth, a Pearson correlation was conducted between DSI and plant height at 5 WAP. In addition, a simple linear regression model was fitted to evaluate the effect of disease severity on plant height using the model:

$$Y = \beta_0 + \beta_1 X + \varepsilon$$

where:

Y = plant height (cm)

X = disease severity index (%)

β_0 = intercept

β_1 = regression coefficient

ε error term

Model fit was evaluated using the coefficient of determination (R^2), adjusted R^2, and F-statistics. Statistical significance at $P \leq 0.05$.

All assumptions of ANOVA (normality and homogeneity of variance) were verified using residual diagnostics.

Results

Occurrence and identification of fungal isolates

Five fungal species were isolated from the sandy loam soil samples and identified based on morphological and microscopic characteristics (Table II). The isolates comprised *Fusarium oxysporum, Trichoderma harzianum, Aspergillus niger, Aspergillus flavus*, and *Penicillium chrysogenum*. The relative occurrence of the isolates varied across species (Fig. 1), with *Aspergillus niger* exhibiting the highest frequency of occurrence, while *Fusarium oxysporum* accounted for approximately 10% of the total isolates.

Table 2
Morphological and microscopic characteristics of fungal isolates obtained from sandy loam soil

Fungal isolate	Colony morphology	Microscopic characteristics	Probable identity
A	White to pink cottony colony	Septate hyphae, sickle-shaped macroconidia	*Fusarium oxysporum*
B	Rapid green colony growth	Branched conidiophores with phialides	*Trichoderma harzianum*
C	Black powdery colony	Radiate conidial heads	*Aspergillus niger*
D	Yellow-green colony	Rough conidiophores with spherical conidia	*Aspergillus flavus*
E	Blue-green colony	Brush-like conidiophores	*Penicillium chrysogenum*

In vitro antagonistic activity against Fusarium oxysporum

Significant differences ($P \leq 0.05$) were observed among fungal antagonists in their ability to inhibit the radial growth of *Fusarium oxysporum* (Figure II). *Trichoderma harzianum* recorded the highest percentage inhibition of radial growth (68%), followed by *Aspergillus niger* (55%) and *Aspergillus flavus* (48%). The control treatment (pathogen alone) showed maximum radial growth (85.0 mm).

Plate 1: Dual culture interaction between *Trichoderma harzianum* and *Fusarium oxysporum* on potato dextrose agar

Plate 2: Dual culture interaction between *Aspergillus niger* and *Fusarium oxysporum* on potato dextrose agar

Plate 3: Dual culture interaction between *Aspergillus flavus* and *Fusarium oxysporum* on potato dextrose agar

Plates show inhibition zones and interaction patterns between antagonist and pathogen after 5 days of incubation at 28°C.

Disease incidence and severity

Disease incidence (DI) and disease severity index (DSI) differed significantly among treatments ($P \leq 0.05$) (Table III). At 5 WAP, plants inoculated with *Fusarium oxysporum* alone recorded the highest disease levels. At an inoculum concentration of 1×10^{10} conidia mL^{-1}, DI and DSI were 92.5% and 80.6%, respectively, compared to 82.0% and 70.4% at 1×10^{8} conidia mL^{-1}. In treatments combining pathogen and antagonists, DI ranged from 24.2% to 60.8%, while DSI ranged from 19.6% to 52.1%. The lowest DI (24.2%) and DSI (19.6%) were recorded in the treatment involving *T. harzianum* at 1×10^{10} conidia mL^{-1}.

The control treatment maintained DI and DSI values of 0% throughout the experimental period. The least significant difference ($LSD_{0.05}$) values were 3.187 for DI and 3.262 for DSI.

Table 3
Effect of fungal antagonists on disease incidence (DI) and disease severity index (DSI) of *Corchorus olitorius*

Treatment	**DI (%)**	**DSI (%)**
Control	0.0	0.0
FO (10^8)	82.0	70.4
FO (10^{10})	92.5	80.6
FO + *T. harzianum* (10^8)	38.5	33.8
FO + *T. harzianum* (10^{10})	24.2	19.6
FO + *A. niger* (10^8)	54.6	46.9
FO + *A. niger* (10^{10})	44.3	39.7
FO + *A. flavus* (10^8)	60.8	52.1
FO + *A. flavus* (10^{10})	51.5	45.3
LSD (P ≤ 0.05)	**3.187*** **	3.262***

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Temporal progression of disease severity

Disease severity index (DSI) increased over time across all treatments involving *F. oxysporum* (Table IV). At 2 WAP, DSI ranged from 10.2% to 38.6% among pathogen-inoculated treatments. At 5 WAP, DSI values increased to between 19.6% and 80.6%. Treatments with antagonists recorded lower DSI values across all sampling periods compared to pathogen-only treatments. At 5 WAP, DSI values were 33.8% and 19.6% for *T. harzianum* at 1×10^8 and 1×10^{10} conidia mL^{-1}, respectively. The $LSD_{0.05}$ values ranged from 2.134 to 2.576 across sampling periods.

Table 4
Temporal progression of disease severity index (DSI, %) in *C. olitorius* under different treatments

Treatment	**2 WAP**	**3 WAP**	**4 WAP**	**5 WAP**
Control	0	0	0	0
FO (10^8)	32.5	51.3	63.7	70.4
FO (10^{10})	38.6	59.8	72.4	80.6
FO + T. harzianum (10^8)	15.4	24.6	30.8	33.8
FO + T. harzianum (10^{10})	10.2	15.8	18.7	19.6
FO + A. niger (10^8)	22.6	35.4	42.8	46.9
FO + A. niger (10^{10})	18.4	29.6	36.7	39.7
FO + A. flavus (10^8)	25.8	40.7	48.9	52.1
FO + A. flavus (10^{10})	21.7	34.5	41.6	45.3
LSD (P ≤ 0.05)	**2.134*** **	**2.311*** **	**2.402*** **	**2.576*** **

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Effect of treatments on plant height

Plant height was significantly affected by treatments at all sampling periods (P ≤ 0.05) (Table V). At 5 WAP, plant height ranged from 16.8 cm to 28.3 cm across treatments. The lowest plant height (16.8 cm) was recorded in plants inoculated with *F. oxysporum* at 1×10^{10} conidia mL^{-1}, while the highest value (28.3 cm) was observed in plants treated with *T. harzianum* at the same inoculum concentration. Intermediate values were recorded across other treatments, including 26.8 cm for A. niger at 1×10^{10} conidia mL^{-1} and 20.2 cm for *A. flavus* at the same concentration. The $LSD_{0.05}$ values ranged from 0.350 to 0.503 across sampling periods.

Table 5
Effect of fungal antagonists on plant height (cm) of *Corchorus olitorius* at different weeks after planting (WAP)

Treatment	2 WAP	3 WAP	4 WAP	5 WAP
Control	10.2	14.2	16.8	18.8
FO (10^8)	8.7	13.8	16.7	18.3
FO (10^{10})	8.5	12.2	15.2	16.8
FO + T. harzianum (10^8)	13.8	18.3	21.1	24.1
FO + T. harzianum (10^{10})	14.9	20.2	24.1	28.3
FO + A. niger (10^8)	13.5	16.8	18.3	22.1
FO + A. niger (10^{10})	14	18.6	20.6	26.8
FO + A. flavus (10^8)	11.8	14.9	17.1	18.2
FO + A. flavus (10^{10})	12.2	15.7	18.1	20.2
LSD (P ≤ 0.05)	**0.350*****	**0.436*****	**0.503*****	**0.463*****

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Effect of treatments on stem girth

Stem girth differed significantly among treatments at all sampling periods (P ≤ 0.05) (Table VI). At 5 WAP, stem girth values ranged from 6.5 mm to 8.6 mm. The lowest value (6.5 mm) was observed in plants inoculated with *F. oxysporum* at 1×10^{10} conidia mL^{-1}, while the highest value (8.6 mm) was recorded in plants treated with *T. harzianum* at the same inoculum level. The $LSD_{0.05}$ values ranged from 0.117 to 0.316.

Table 6
Effect of fungal antagonists on stem girth (mm) of *Corchorus olitorius* at different weeks after planting (WAP)

Treatment	2 WAP	3 WAP	4 WAP	5 WAP
Control	3.6	4.8	6.0	7.0
FO (10^8)	3.2	4.6	5.8	6.8
FO (10^{10})	3.4	4.4	5.5	6.5
FO + T. harzianum (10^8)	4.8	6.0	6.8	7.5
FO + T. harzianum (10^{10})	5.0	6.4	7.1	8.6
FO + A. niger (10^8)	4.2	5.3	6.5	7.4
FO + A. niger (10^{10})	4.5	5.8	6.6	8.2
FO + A. flavus (10^8)	3.7	4.9	6.1	7.2
FO + A. flavus (10^{10})	4.2	5.5	6.6	7.8
LSD (P ≤ 0.05)	**0.219*** **	**0.117*** **	**0.127*** **	**0.316*** **

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Effect of treatments on leaf length

Leaf length varied significantly among treatments (P ≤ 0.05) (Table VII). At 5 WAP, leaf length ranged from 5.5 cm to 8.7 cm. The lowest value (5.5 cm) was recorded in plants inoculated with *F. oxysporum* at 1×10^{10} conidia mL^{-1}, while the highest value (8.7 cm) was observed in plants treated with *T. harzianum* at the same inoculum concentration. The $LSD_{0.05}$ values ranged from 0.267 to 0.278.

Table 7
Effect of fungal antagonists on leaf length (cm) of *Corchorus olitorius* at different weeks after planting (WAP)

Treatment	2 WAP	3 WAP	4 WAP	5 WAP
Control	4.2	4.7	6.0	6.5
FO (10^8)	3.87	4.5	5.9	6.0
FO (10^{10})	3.7	4.4	5.2	5.5
FO + T. harzianum (10^8)	5.1	6.4	7.7	8.1
FO + T. harzianum (10^{10})	5.3	6.6	8.1	8.7
FO + A. niger (10^8)	4.6	5.4	6.2	7.1
FO + A. niger (10^{10})	5.1	5.6	7.1	7.5
FO + A. flavus (10^8)	4.3	4.9	5.8	6.1
FO + A. flavus (10^{10})	4.4	5.5	6.6	7.1
LSD (P ≤ 0.05)	**0.267*** **	**0.278*** **	**0.278*** **	0.271***

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Effect of treatments on leaf width

Significant treatment effects (P ≤ 0.05) were observed for leaf width among treatments (Table VIII). At 5 WAP, leaf width ranged from 2.9 cm to 4.5 cm. The lowest value (2.9 cm) occurred in plants inoculated with *F. oxysporum* at 1×10^{10} conidia mL^{-1}, while the highest value (4.5 cm) was recorded in plants treated with *T. harzianum* at the same inoculum level. The $LSD_{0.05}$ values ranged from 0.160 to 0.201.

Table 8
Effect of fungal antagonists on leaf width (cm) of *Corchorus olitorius* at different weeks after planting (WAP)

Treatment	**2 WAP**	**3 WAP**	**4 WAP**	**5 WAP**
Control	2.3	2.6	3.0	3.5
FO (10^8)	2.2	2.5	2.9	3.1
FO (10^{10})	2.0	2.3	2.7	2.9
FO + T. harzianum (10^8)	2.6	3.1	3.5	4.1
FO + T. harzianum (10^{10})	2.5	3.4	4.0	4.5
FO + A. niger (10^8)	2.4	2.9	3.2	3.7
FO + A. niger (10^{10})	2.3	3.0	3.4	4.2
FO + A. flavus (10^8)	2.3	2.8	3.2	3.6
FO + A. flavus (10^{10})	2.3	2.9	3.2	3.9
LSD (P ≤ 0.05)	**0.160*** **	**0.173*** **	**0.175*** **	**0.201*** **

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Effect of treatments on the number of leaves

The number of leaves per plant differed significantly (P ≤ 0.05) among treatments (Table IX). At 5 WAP, leaf number ranged from 6.7 to 19.3 leaves per plant. The lowest value (6.7) was observed in plants inoculated with *F. oxysporum* at 1 × 10^{10} conidia mL^{-1}, while the highest value (19.3) was recorded in plants treated with *T. harzianum* at the same inoculum concentration. The $LSD_{0.05}$ values ranged from 1.081 to 1.625.

Table 9
Effect of fungal antagonists on the number of leaves per plant of *Corchorus olitorius* at different weeks after planting (WAP)

Treatment	2 WAP	3 WAP	4 WAP	5 WAP
Control	7.3	10.0	10.7	12.7
FO (10^8)	5.7	7.7	7.7	10.0
FO (10^{10})	6.7	7.7	9.3	6.7
FO + T. harzianum (10^8)	10.0	13.0	14.7	15.7
FO + T. harzianum (10^{10})	10.7	15.7	17.0	19.3
FO + A. niger (10^8)	8.0	11.3	12.3	14.3
FO + A. niger (10^{10})	10.0	13.7	15.3	16.7
FO + A. flavus (10^8)	7.3	11.0	11.3	12.0
FO + A. flavus (10^{10})	7.7	12.3	12.3	14.3
LSD (P ≤ 0.05)	**1.081*****	**1.300*****	**1.333*****	1.625***

FO = *F. oxysporum*, 10^8 = 10^8 conidia mL^{-1} inoculum load, 10^{10} = 10^{10} conidia mL^{-1} inoculum load, *** = significance at P ≤ 0.001

Relationship between disease severity and plant height

Pearson correlation analysis revealed a strong negative association between disease severity index (DSI) and plant height at 5 WAP (Table X). The correlation coefficient (*r*) between DSI and plant height was −0.957, which was highly significant (P ≤ 0.001).

Table 10
Pearson correlation matrix between disease severity (DSI) and plant height (5 WAP)

Parameter	DSI (%)	Plant height (cm)
DSI (%)	1.000	-0.957***
Plant height (cm)	-0.957***	1.000

Values represent (*r*); *** = significance at P ≤ 0.001

Linear regression analysis further showed that disease severity significantly influenced plant height (Table XI). The regression model explained a substantial proportion of the variability in plant height (R^2 = 0.916, P ≤ 0.001).

Table 11
Linear regression analysis of plant height as influenced by disease severity (% WAP)

Parameter	Estimate	Standard Error	t-value	P-value
Intercept (β_0)	33.412	1.287	25.95	≤ 0.001
DSI (%) (β_1)	-0.204	0.015	-13.60	≤ 0.001
R^2	0.916	-	-	-
Adjusted R^2	0.904	-	-	-
F-value	184.96	-	-	≤ 0.001
*** = significance at P ≤ 0.001				

Discussion

Antagonistic efficacy of fungal isolates

The present study demonstrated that all evaluated fungal antagonists inhibited the growth of *Fusarium oxysporum*, with *Trichoderma harzianum* exhibiting the highest suppression (68%). This level of inhibition falls within the moderate-to-high efficacy range reported globally for *Trichoderma*-based biocontrol systems. Recent studies indicate that inhibition of *Fusarium spp*. by *T. harzianum* typically ranges between 50% and over 80%, depending on strain specificity, pathogen virulence, and environmental conditions (Afifi et al., 2019; Chen et al., 2021; Palmieri et al., 2022; Shin et al., 2023). The observed variability among fungal antagonists highlights the strain- and species-dependent nature of biological control efficacy (Yao et al., 2023; Jaiswal & Tiwari, 2024). While *A. niger* and *A. flavus* demonstrated measurable inhibition, their comparatively lower performance aligns with reports that non-*Trichoderma* fungi often exhibit limited antagonistic breadth against vascular wilt pathogens due to narrower modes of action (Mundim et al., 2022; Yao et al., 2023; Jaiswal & Tiwari, 2024). Importantly, the inhibition level recorded in this study suggests that the indigenous *T. harzianum* isolate possesses substantial antagonistic capacity, although it does not represent the upper efficacy threshold reported under optimised or strain-selected conditions. This underscores the importance of strain selection and optimisation in biocontrol development (Harman et al., 2021; Palmieri et al., 2022).

Mechanistic basis of pathogen suppression

The superior antagonistic performance of *T. harzianum* can be attributed to its multifunctional mechanisms of action. *Trichoderma* species suppress *Fusarium* through a combination of mycoparasitism, antibiosis, competition, and induced plant resistance (Harman et al., 2021; Yao et al., 2023; Mulatu et al., 2023; Jaiswal & Tiwari, 2024; Wang et al., 2024). At the biochemical level,

Trichoderma produces cell wall-degrading enzymes, such as chitinases, glucanases, and proteases, that facilitate the direct degradation of *Fusarium* hyphae (Chen et al., 2021; Mulatu et al., 2023; Wang et al., 2024). Concurrently, the secretion of secondary metabolites and volatile organic compounds contributes to antibiosis and inhibition of pathogen growth (Chen et al., 2021; Palmieri et al., 2022; Mulatu et al., 2023; Wang et al., 2024). These mechanisms are particularly effective against *Fusarium*, which relies on enzymatic degradation of host tissues during infection (Michielse & Rep, 2009; Dean et al., 2012; Awal et al., 2024). In addition to direct antagonism, *T. harzianum* is known to enhance host defence systems through induced systemic resistance (ISR) (Harman et al., 2021; Mulatu et al., 2023; Wang et al., 2024). Experimental evidence shows that *Trichoderma* application can stimulate antioxidant enzyme activity and defence-related pathways, thereby increasing plant tolerance to *Fusarium* infection (Mundim et al., 2022; Yao et al., 2023; Jaiswal & Tiwari, 2024). The comparatively lower efficacy of *Aspergillus* species observed in this study may reflect their reliance on indirect mechanisms such as organic acid production and nutrient competition, which are less effective against aggressive vascular pathogens. This distinction reinforces the importance of selecting antagonists with multiple complementary modes of action.

Disease suppression under controlled conditions

The substantial reduction in disease incidence and severity observed under screenhouse conditions confirms the functional effectiveness of fungal antagonists in vivo. The approximately 70–75% reduction in disease severity achieved by *T. harzianum* is consistent with recent greenhouse and controlled-environment studies reporting significant suppression of *Fusarium* wilt across diverse crops (Chen et al., 2021; Afifi et al., 2019). Such levels of suppression indicate successful rhizosphere colonisation and competitive exclusion of the pathogen. However, disease suppression in controlled environments is often enhanced by reduced microbial competition and environmental variability. Consequently, the magnitude of suppression observed in this study may represent an upper estimate relative to field conditions (Palmieri et al., 2022). The dose-dependent response observed further suggests that inoculum density influences colonisation efficiency and antagonistic performance (Afifi et al., 2019; Yao et al., 2023; Jaiswal & Tiwari, 2024). Higher inoculum concentrations likely facilitated the rapid establishment of *T. harzianum* in the rhizosphere, thereby enhancing both pathogen suppression and plant protection.

Growth-promoting effects and plant-microbe interactions

Beyond disease suppression, the significant improvements in plant growth parameters highlight the dual role of *T. harzianum* as both a biocontrol agent and a plant growth-promoting fungus (PGPF). Enhanced plant height, stem girth, and leaf development observed in this study are consistent with reports that *Trichoderma* improves nutrient uptake, root architecture, and phytohormone production (Palmieri et al., 2022; Mulatu et al., 2023; Wang et al., 2024). These growth-promoting effects are often linked to the production of auxin-like compounds, enhanced nutrient solubilization, and improved root colonisation and rhizosphere modification (Chen et al., 2021; Harman et al., 2021). The strong inverse relationship

between disease severity and plant growth further supports the integrated role of *T. harzianum* in both disease mitigation and plant performance enhancement. Similar dual-function behaviour has been widely reported in recent studies, where *Trichoderma* simultaneously suppresses pathogens and enhances plant physiological resilience (Palmieri et al., 2022; Mulatu et al., 2023; Wang et al., 2024).

Comparative performance and implications for biocontrol selection

The comparative evaluation conducted in this study provides clear evidence that fungal antagonists differ significantly in their effectiveness against *Fusarium oxysporum*. The consistent superiority of *T. harzianum* across in vitro and in vivo assessments aligns with its global recognition as one of the most reliable microbial biocontrol agents (Harman et al., 2021; Yao et al., 2023; Jaiswal & Tiwari, 2024). Recent advances in biocontrol research emphasise the importance of strain-specific selection, formulation technology, and ecological adaptability in determining field success (Palmieri et al., 2022). For instance, emerging bioformulation approaches, including carrier-based and encapsulated systems, have been shown to enhance the stability and efficacy of *Trichoderma*-based products under field conditions (Palmieri et al., 2022; Villavicencio-Vásquez et al., 2025). In contrast, the relatively lower performance and biosafety concerns associated with *A. flavus* limit its practical application. The potential for aflatoxin production presents a significant risk, particularly in food crops, and necessitates careful consideration in biocontrol selection (Amaike & Keller, 2011).

Limitations and sources of experimental bias

Despite the promising findings, several limitations must be acknowledged. First, fungal identification was based solely on morphological characteristics, which may not provide sufficient taxonomic resolution. Molecular identification using ITS sequencing would enhance accuracy and reproducibility. Second, the use of sterilised soil, while necessary to isolate treatment effects, removes native microbial communities and may artificially enhance antagonist performance. This creates a soil sterilisation bias, potentially overestimating efficacy relative to natural field conditions. Third, the study was conducted under controlled screenhouse conditions, which do not fully capture environmental variability, microbial competition, and abiotic stress factors present in field systems. Finally, the limited number of replicates, although statistically acceptable, may reduce the robustness of treatment comparisons under more variable conditions.

Implications for sustainable crop protection

The findings of this study contribute to the growing body of evidence supporting microbial biocontrol as a sustainable alternative to chemical fungicides. The demonstrated efficacy of *T. harzianum* reinforces its potential for integration into integrated disease management (IDM) systems. Given increasing concerns regarding fungicide resistance, environmental contamination, and soil health degradation, microbial-based interventions represent a critical component of sustainable crop production. The ability

of *Trichoderma* to function as both a disease suppressor and growth promoter further enhances its value in low-input and environmentally conscious agricultural systems.

Future research directions

To enhance the applicability and scientific robustness of the findings, future studies should therefore focus on:

1. Molecular identification and strain characterisation of fungal isolates.
2. Field validation across diverse agroecological zones.
3. Evaluation under non-sterile soil conditions to capture ecological interactions.
4. Mechanistic studies involving enzyme assays and metabolite profiling.
5. Development and optimisation of bioformulations.

Conclusions

This study demonstrates that fungal antagonists can effectively suppress *Fusarium oxysporum* and improve the growth performance of *Corchorus olitorius* under controlled conditions. Among the evaluated isolates, *Trichoderma harzianum* consistently exhibited the highest antagonistic activity, achieving substantial inhibition of pathogen growth in vitro and marked reductions in disease incidence and severity in vivo. In addition to disease suppression, *T. harzianum* significantly enhanced plant growth parameters, indicating its dual role as a biocontrol and plant growth-promoting agent. In contrast, *Aspergillus niger* showed moderate efficacy, while *Aspergillus flavus* demonstrated comparatively lower performance and presents biosafety concerns due to its toxigenic potential. Overall, the integration of in vitro and screenhouse evaluations confirms the robustness of *T. harzianum* as a promising candidate for biological management of *Fusarium* wilt in *C. olitorius*. However, the findings are constrained by controlled experimental conditions and require validation under field environments.

Declarations

Ethics approval and consent to participate

This study did not involve human participants, human data, or animals. The Plant material used in this study was cultivated *Corchorus olitorius*. The seeds were commercially sourced and cultivated under controlled screenhouse conditions at the Department of Agronomy, University of Ilorin, Nigeria. The collection and use of the plant material complied with relevant institutional, national, and international guidelines for plant research. No wild plant species were collected, and no specific permits or licenses were required for the use of the cultivated plant material in this study.

Consent for publication

Not applicable

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Authors’ contributions

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Review & editing: James Ukwumonu Yahaya, Oluyemisi Bolajoko Fawole, Damilola Abigael Ikuoponiyi and Ahmed Oladimeji.

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Availability of data and materials

All data generated or analysed during this study are included in this manuscript. Additional information related to the datasets used and analysed during the current study is available from the corresponding author upon reasonable request.

Clinical trial number

Not applicable

Competing interests

The authors declare that there are no competing interests, financial or non-financial, that could have influenced the work reported in this manuscript.

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Plates

Plates 1 to 3 available in the Supplementary Files section.

Figures

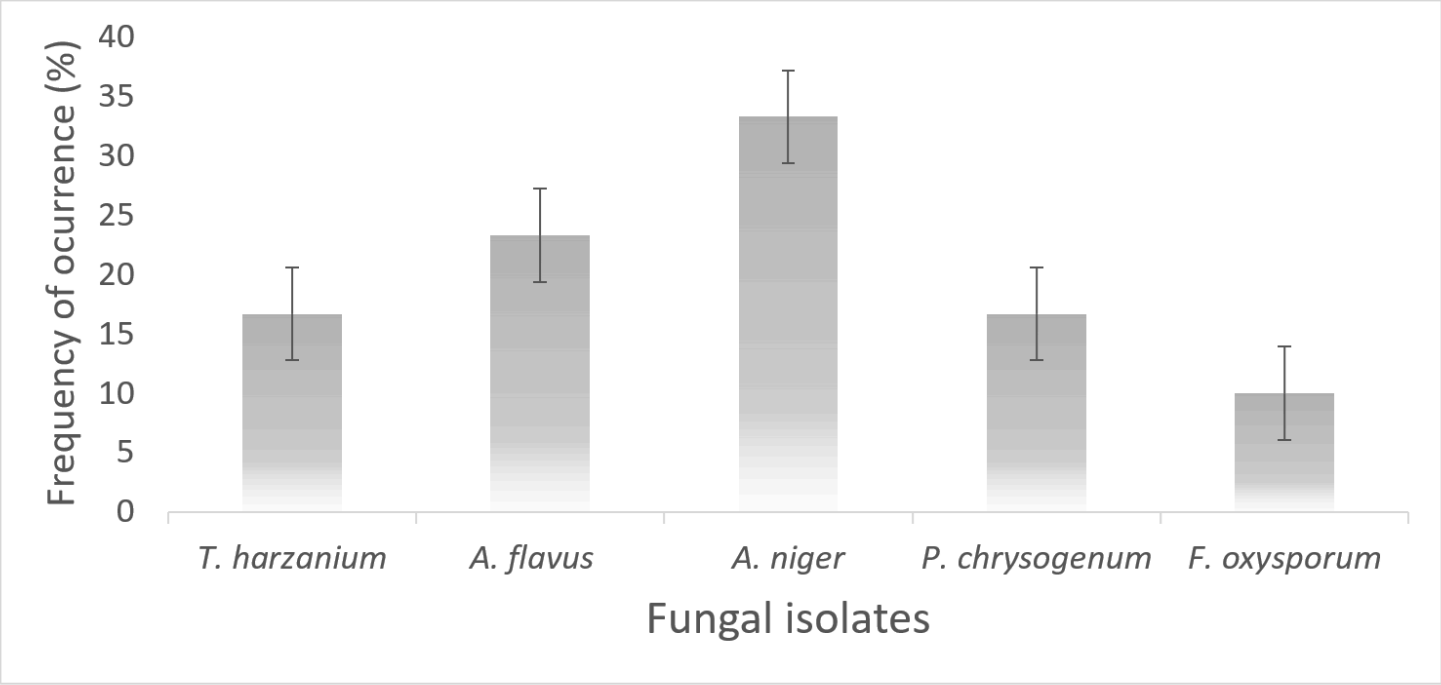

Figure 1

Relative occurrence (%) of fungal isolates recovered from sandy loam soil

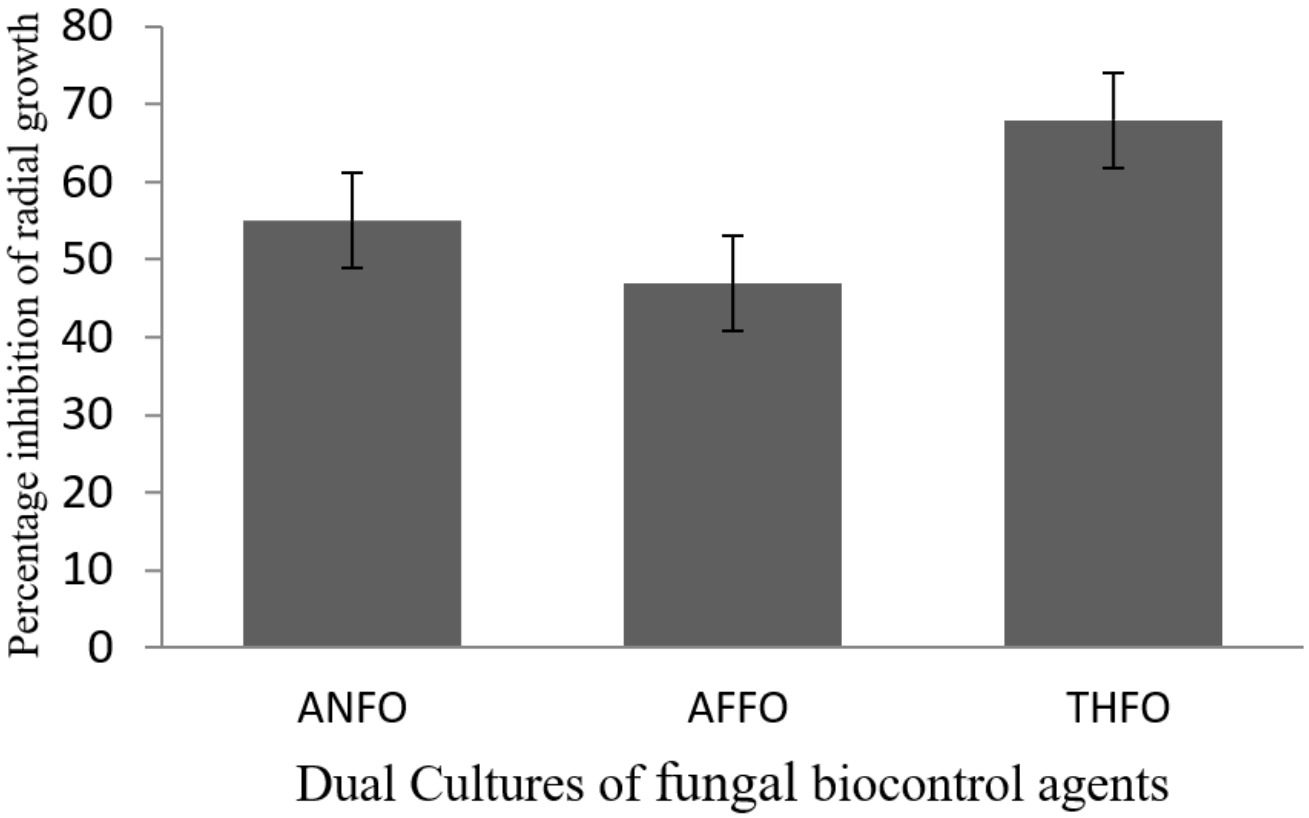

Figure 2

In vitro inhibition of Fusarium oxysporumby fungal antagonists expressed as percentage inhibition of radial growth (PIRG)

ANFO = *Aspergillus niger* + *Fusarium oxysporum*, AFFO = *Aspergillus flavus* + *Fusarium oxysporum*, THFO = *Trichoderma harzanium* + *Fusarium oxysporum*

Supplementary Files

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