

RESEARCH PAPER

OPEN ACCESS

Biocontrol potential of indigenous *Trichoderma* isolates against *Fusarium* wilt of cotton in Tanzania: *In vitro* screening and screen-house validation

Fauzia Khalid Mpiganzila^{*1,2}, Alfonce Leonard², Lidia Munuo¹, Ernest Mbega¹,
Agatha Aloyce¹

¹*School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania*

²*Tanzania Agricultural Research Institute (TARI), Mwanza Centre, Mwanza, Tanzania*

Key words: Biological control, *Trichoderma*, *Fusarium oxysporum*, Cotton wilt, AUDPC

Received: August 19, 2026 **Accepted:** August 29, 2026 **Published:** September 07, 2026

DOI: <https://dx.doi.org/10.12692/ijb/29.3.44-61>

ABSTRACT

Fusarium wilt constrains cotton production in Tanzania. This study evaluated indigenous cotton-rhizosphere microorganisms as biological control candidates through sequential *in vitro* and screenhouse assays. Thirty-six rhizosphere soil samples collected from cotton farms in Magu, Kwimba and Misungwi districts yielded 27 microbial isolates. Preliminary screening selected five candidates: two putative members of the *Trichoderma harzianum* species complex (T1 and T2), *Bacillus subtilis* (T3), *Bacillus amyloliquefaciens* (T4) and a T3+T4 consortium (T5). In dual-culture assays against *Fusarium oxysporum* EF1, T2 and T1 produced the greatest Day-7 inhibition of radial growth at 65.9% and 61.2%, respectively, compared with 29.5–36.4% for the *Bacillus*-based treatments. T1 and T2 were subsequently evaluated in a six-week screenhouse trial using cotton cultivar UKM 08. Relative to the pathogen control, T1 and T2 reduced mean area under the disease progress curve by 74.6% and 43.2%, respectively. At Week 6, both treatments significantly reduced normalized disease severity, although Holm-adjusted comparisons of disease incidence were not significant. T2 showed slightly greater inhibition *in vitro*, whereas T1 produced a larger numerical reduction in cumulative disease, but the isolates did not differ significantly in direct comparisons. These findings demonstrate that *in vitro* antagonism may not fully predict plant-associated performance and support sequential laboratory and host-based screening. Both isolates require field validation before practical recommendation.

***Corresponding author:** Fauzia Khalid Mpiganzila ✉ mpiganzilaf@nm-aist.ac.tz

^{*} <https://orcid.org/0009-0003-3533-991X>

Co-authors:

Alfonce Leonard: <https://orcid.org/0000-0003-2242-2389>

Ernest Mbega: <https://orcid.org/0000-0001-8812-2624>

Agatha Aloyce: <https://orcid.org/0009-0000-4395-0442>

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important fibre and cash crop that supports textile industries and rural livelihoods worldwide (Tarazi *et al.*, 2020; Javed *et al.*, 2025). In Tanzania, it is a strategic commercial crop, particularly in the Lake Zone. National seed-cotton production increased from approximately 149,000 tonnes in 2024/2025 to about 222,000 tonnes in 2025/2026, reflecting continued investment in production and extension services (Tanzania Cotton Board [TCB], 2026). Cotton also contributes substantially to smallholder household income, and improved production practices could strengthen its socioeconomic benefits (Mfinanga *et al.*, 2025).

Fusarium wilt, primarily associated with *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is an important soil-borne disease of cotton. The pathogen invades roots and colonizes vascular tissues, causing chlorosis, wilting, vascular discoloration, stunting, defoliation and, in severe cases, plant death (Davis *et al.*, 2006; Michielse and Rep, 2009; Javed *et al.*, 2025). Its long-lived survival structures enable it to persist in infested soils, making management difficult.

Disease development varies with cotton genotype, pathogen lineage, environmental conditions and interactions with other soil organisms. Root-knot nematodes influence some FOV pathotypes, whereas highly virulent lineages such as race 4 can cause severe disease independently of nematode association (Bell *et al.*, 2017; Zhou *et al.*, 2026). Therefore, yield-loss estimates and environmental thresholds should be interpreted according to the pathogen lineage, cultivar and production environment. Variation in the responses of Tanzanian cotton genotypes further demonstrates the importance of host resistance (Ndole *et al.*, 2023).

Although resistant cultivars, sanitation, crop rotation and chemical treatments are used, pathogen persistence and diversity, together with the environmental and economic limitations of chemical

inputs, create a need for complementary management approaches (Cianchetta and Davis, 2015; Sanogo and Zhang, 2016; Javed *et al.*, 2025).

Biological control using antagonistic microorganisms is a promising complementary strategy. *Trichoderma* and *Bacillus* species can suppress soil-borne fungal pathogens through competition for nutrients and space, production of antifungal metabolites and hydrolytic enzymes, mycoparasitism and stimulation of host defence responses (Harman *et al.*, 2004; Vinale *et al.*, 2008; Woo *et al.*, 2023). Recent studies have demonstrated that *T. harzianum*-based treatments can reduce *Fusarium* wilt in cotton, while rhizosphere-derived *Bacillus* isolates can inhibit FOV *in vitro* and suppress disease in greenhouse-grown plants (Aslam *et al.*, 2025; Javed *et al.*, 2025). Indigenous rhizosphere microorganisms are suitable candidates for local screening because they originate from the target cropping environment; however, their ecological fitness, persistence and superiority over commercial or non-indigenous strains must be demonstrated experimentally (Druzhinina *et al.*, 2011; Mukherjee *et al.*, 2013). Although *Fusarium* wilt and genotype-dependent responses have been documented in Tanzanian cotton, the biocontrol potential of indigenous cotton-rhizosphere microorganisms remains poorly understood (Ndole *et al.*, 2023). Moreover, inhibition on agar does not account for interactions among the antagonist, pathogen, plant and soil, making sequential laboratory and plant-based evaluation necessary.

Accordingly, this study evaluated microbial isolates recovered from cotton-rhizosphere soils in Magu, Kwimba and Misungwi districts of Tanzania. The objectives were to: (i) isolate and characterize native rhizosphere microorganisms; (ii) assess their *in vitro* antagonistic activity against *F. oxysporum* EF1 using percent inhibition of radial growth (PIRG) and inhibition-zone measurements; (iii) evaluate the effects of the two most consistently antagonistic *Trichoderma* isolates, T1 and T2, on *Fusarium* wilt development in a screenhouse experiment using disease severity, incidence, normalized severity and

area under the disease progress curve (AUDPC); and (iv) descriptively compare the performance rankings of T1 and T2 between the *in vitro* and screenhouse assays. The *in vitro* phase identified the strongest and most persistent antagonists for plant-based evaluation. We hypothesized that T1 and T2 would reduce *Fusarium* wilt development relative to the pathogen control under the tested screenhouse conditions.

MATERIALS AND METHODS

Study sites

Two study settings were used for the sequential phases of this work. Rhizosphere soil sampling and microbial isolation were conducted in Magu, Kwimba

and Misungwi districts, major cotton-producing areas of Mwanza Region, Tanzania (Fig. 1A). The region has a tropical climate with short rains mainly from October to December and longer rains from March to May, accompanied by seasonal temperature variation (Esmail and Oelbermann, 2022). Misungwi District receives approximately 600–1,000 mm of annual rainfall and has soils ranging from imperfectly drained sands, sandy clays and cracking clays to well-drained gravelly sandy loams and sandy clay loams (United Republic of Tanzania, 2017). These published characteristics provide regional context because site-specific soil properties and climatic conditions were not measured; therefore, their influence on *Fusarium* persistence was not evaluated.

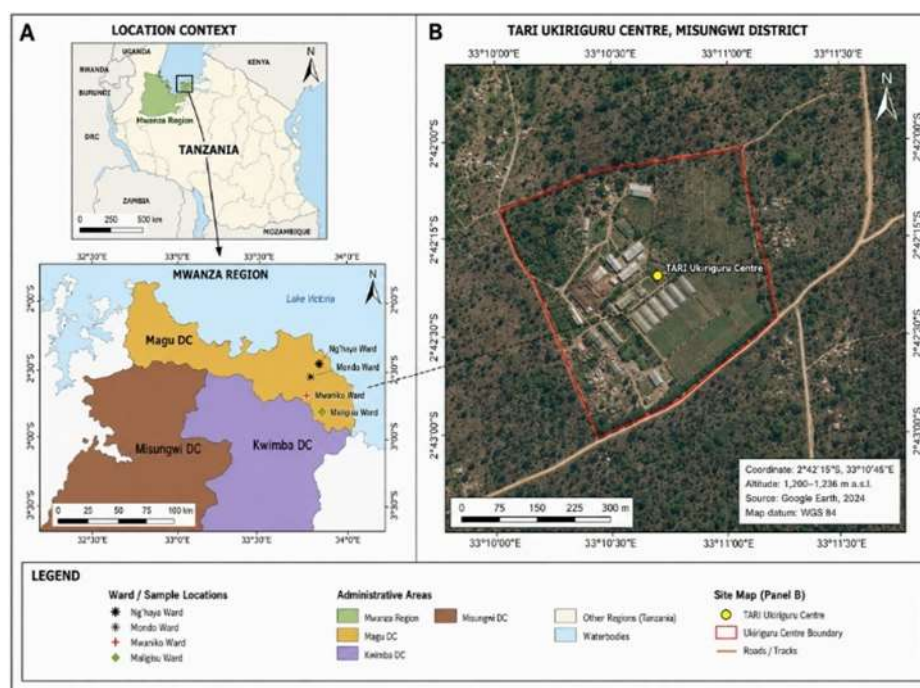


Fig. 1. Study sites used in the two sequential phases of this work: (A) Rhizosphere soil-sampling areas across Magu, Kwimba, and Misungwi districts in Mwanza Region, Tanzania; (B) Location of the screenhouse study site at TARI Ukiriguru Centre, Misungwi District, Mwanza Region, Tanzania

The screenhouse trial was conducted at the Tanzania Agricultural Research Institute, Ukiriguru Centre, Misungwi District (approximately 2°24'15" S, 33°10'45" E; 1,200–1,236 m a.s.l.; Fig. 1B). The screenhouse protected plants from direct rainfall and external disturbances, while irrigation and routine maintenance were applied uniformly throughout the experiment.

Rhizosphere soil sampling and microbial isolation

A stratified sampling approach was used in Magu, Kwimba and Misungwi districts of Mwanza Region, Tanzania. One established cotton-producing ward with a reported history of *Fusarium* wilt was purposively selected from each district: Ng'haya, Maligisu and Mwaniko, respectively. Within each

ward, three eligible farms were randomly selected and coded MAG-F1–F3, KWI-F1–F3 and MIS-F1–F3. Farm-level coordinates were not retained in the archived records; therefore, Fig. 1A presents only ward- and district-level locations.

Leaf chlorosis, wilting and vascular discoloration were used to support farm selection but not as diagnostic confirmation of *Fusarium oxysporum* (Davis *et al.*, 2006; Ndole *et al.*, 2023). At each farm, four separate rhizosphere samples were collected from healthy cotton plants at spatially distinct points and a depth of 0–20 cm using a sterile soil auger. Samples were not composited. Twelve samples were collected per district, giving 36 samples in total. They were transported at approximately 4°C and processed separately within three days under aseptic conditions.

For microbial isolation, 1 g of each soil sample was suspended in 9 mL of sterile distilled water to obtain a 10^{-1} suspension, followed by ten-fold serial dilution to 10^{-6} . For bacterial isolation, 0.1 mL of the 10^{-4} and 10^{-5} dilutions was spread on Nutrient Agar, with three replicate plates per dilution and sample, and incubated at 28°C for 24 h. Morphologically distinct, well-separated colonies were selected according to size, shape, margin, elevation, pigmentation and surface appearance. They were purified by repeated streaking and characterized by colony morphology and Gram reaction. For fungal isolation, 0.1 mL of the 10^{-3} and 10^{-4} dilutions was spread on Potato Dextrose Agar supplemented with chloramphenicol at 100 mg L⁻¹. Three replicate plates were prepared per dilution and sample and incubated at 25°C for five days. Distinct, spatially separated colonies were transferred to fresh medium and purified by repeated hyphal-tip subculturing (Barnett and Hunter, 1998).

Purified bacterial and fungal isolates were maintained on agar slants. A total of 27 morphologically distinct isolates, comprising 18 bacteria and nine fungi, were recovered and characterized by colony morphology, growth and microscopic features; bacteria were additionally characterized by Gram reaction. All

isolates were evaluated for plant-beneficial traits and preliminary antagonistic activity against *F. oxysporum* EF1 using a dual-culture assay. Five isolates, designated T1–T5, were advanced based on distinct morphology, vigorous growth, relevant functional traits and comparatively strong antagonistic activity. Isolates producing inhibition zones greater than 10 mm were considered suitable for advancement. The remaining isolates showed poor growth and/or negligible inhibition and were not evaluated further.

Morphological and molecular identification

Fungal isolates T1 and T2 were cultured on Potato Dextrose Agar (PDA) at 28°C for seven days and characterized by colony and microscopic morphology. Lactophenol cotton blue preparations revealed rapid mycelial growth, septate hyphae, branched conidiophores, phialides and abundant green conidia characteristic of *Trichoderma* (Yao *et al.*, 2023).

Bacterial isolates T3 and T4 were cultured on Nutrient Agar at 37°C for 24 h and characterized by colony morphology, Gram reaction and endospore formation (Logan and De Vos, 2015). T4 was also evaluated for lipolytic activity on tributyrin agar (Duan *et al.*, 2021).

The test pathogen, *Fusarium oxysporum* EF1, was obtained from the TARI Ukiriguru Laboratory culture collection. It had previously been isolated from symptomatic cotton and characterized by partial sequencing of the translation elongation factor 1- α (*tef1- α*) gene, with the sequence deposited in GenBank under accession MK482288.1. Isolation, DNA extraction and sequencing were therefore not repeated. Before use, EF1 was subcultured on PDA and examined for purity and viability. Because partial *tef1- α* sequence data do not confirm host specificity, the isolate was designated *F. oxysporum*.

Genomic DNA was extracted from pure T1 and T2 cultures using the CTAB method (Sambrook and Russell, 2001). A partial *tef1- α* region was amplified

using primers EF1-728F (5'-CATCGAGAAGTTCGAGAAGG-3') and EF1-986R (5'-TACTTGAAGGAACCCTTACC-3') (Carbone and Kohn, 1999). Each 25- μ L reaction contained 12.5 μ L of 2 $\times$ PCR Master Mix, 1.0 μ L of each primer (10 μ M), 1.0 μ L of DNA (approximately 50 ng μ L⁻¹) and 9.5 μ L of nuclease-free water. Amplification consisted of 95°C for 5 min; 35 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 1 min; and a final extension at 72°C for 7 min. Each run included positive and no-template controls. Amplicons were separated on 1% agarose gels in 1 $\times$ TAE buffer at 120 V for 70 min alongside a 1-kb DNA ladder. The manufacturer and catalogue number of the Master Mix and the strain identity of the historical positive control were not retained in the archived records.

Amplicons were purified and sequenced bidirectionally by Sanger sequencing. Forward and reverse chromatograms were inspected, ambiguous terminal regions were removed and consensus sequences were assembled and compared with GenBank sequences using BLASTn. The archived records did not retain the processing software and version, numerical quality threshold, BLASTn search date or database release, or fixed identity and query-coverage thresholds. Taxonomic interpretation therefore considered sequence identity, query coverage, concordance among the closest informative matches and isolate morphology.

Study-specific GenBank accessions were not assigned, and closest-reference accessions are not reported

because the original BLASTn output was unavailable for verification. Based on the combined evidence, T1 and T2 were conservatively assigned to the putative *Trichoderma harzianum* species complex. Assignment to *T. harzianum* sensu stricto was not made because only one partial locus was analysed without multilocus phylogenetic analysis.

T3 and T4 were identified by species-specific endpoint PCR. T3 was tested for *Bacillus subtilis* using *ytcp*-F (5'-GCTTACGGGTATCCCGC-3') and *ytcp*-R (5'-CCGACCCCATTTTCAGACATATC-3'), which amplify an approximately 460-bp region of *ytcp* (Kwon *et al.*, 2009). T4 was tested for *Bacillus amyloliquefaciens* using the *pheS*-targeted primers spBamyphes-171F1 (5'-TCTCGTCGTSGACCGCAA-3') and 353R1 (5'-GATCCAGCCTGTTTTTTCG-3'), which produce a 202-bp amplicon (Huang *et al.*, 2017). Reaction composition and cycling conditions were as described above. Each assay included positive-control DNA from a previously confirmed target species and a no-template control; strain identifiers of the historical positive controls were not retained. Products were separated on 1.5% agarose gels in 1 $\times$ TAE, stained with ethidium bromide and visualized under UV light. Identification required a single band of the expected size in the isolate and positive control and no amplification in the negative control. T3 produced the expected *ytcp* product and was identified as *B. subtilis*, whereas T4 produced the expected *pheS* product and was identified as *B. amyloliquefaciens*. Identification results are summarized in Table 1.

Table 1. Molecular identification and origin of the microbial isolates used in this study

Isolate	Taxonomic identification	Molecular method	GenBank accession of study isolate	Closest BLASTn reference accession	Reported BLAST similarity (%)	Source
T1	Putative <i>T. harzianum</i> species complex	Partial tef1- α sequencing + BLASTn	Not assigned	KC576690	98	Kwimba
T2	Putative <i>T. harzianum</i> species complex	Partial tef1- α sequencing + BLASTn	Not assigned	KX254451	99	Misungwi
T3	<i>Bacillus subtilis</i>	Species-specific endpoint PCR targeting <i>ytcp</i>	Not applicable	Not applicable	Not applicable	Magu
T4	<i>Bacillus amyloliquefaciens</i>	Species-specific endpoint PCR targeting <i>pheS</i>	Not applicable	Not applicable	Not applicable	Kwimba
T5	T3 + T4 bacterial consortium	1:1 (v/v) mixture	Not applicable	Not applicable	Not applicable	Laboratory prepared
EF1	<i>Fusarium oxysporum</i>	Previous partial tef1- α sequencing	MK482288.1	Not applicable	Not applicable	TARI Ukiriguru culture collection

***In vitro* antagonism assay**

Antagonistic activity against *Fusarium oxysporum* EF1 was evaluated using a dual-culture assay on PDA (Dennis and Webster, 1971). The experiment comprised seven treatments: T1, T2, T3, T4, the T3+T4 consortium (T5), an untreated pathogen control consisting of EF1 alone, and a carbendazim-treated control ($2 \mu\text{g mL}^{-1}$). A 5-mm mycelial plug of *F. oxysporum* EF1 was placed 1 cm from the edge of each plate. Fungal antagonists were inoculated as opposing 5-mm plugs approximately 6 cm from EF1, whereas bacterial isolates were applied as opposing streaks. T5 consisted of T3 and T4 co-inoculated at a 1:1 ratio. Each treatment comprised three replicate plates per run, and the experiment was conducted twice. Plates and inocula were prepared separately for each run and treatments were randomized independently. Plates were incubated at 25°C for 7 days, and EF1 radial growth toward the antagonist was measured daily from the edge of the original inoculum plug to the colony interaction front. Once colonies contacted or antagonist overgrowth occurred, the last measurable pathogen radius at the interaction interface was recorded, and overgrowth was documented qualitatively. Results from both independent runs contributed to the descriptive treatment summaries; the runs were not treated as six replicates from a single experimental occasion. Carbendazim served as an efficacy benchmark rather than a geometrically equivalent dual culture treatment; therefore, inhibition-zone measurements were considered not applicable for this control. Percent inhibition of radial growth (PIRG) was calculated following Skidmore and Dickinson (1976):

$$\text{PIRG (\%)} = [(R_1 - R_2) / R_1] \times 100$$

where:

R_1 is the radial growth of *F. oxysporum* EF1 in the untreated pathogen control; and

R_2 is the radial growth of *F. oxysporum* EF1 in the presence of the antagonist.

Inhibition-zone width was measured in millimetres along the line connecting the pathogen and antagonist

inoculation points, as the shortest distance between the advancing margins of *F. oxysporum* and the antagonist. When colonies met or antagonist overgrowth occurred, the inhibition-zone value was recorded as 0 mm; inhibition-zone measurements were not applicable to the carbendazim-amended control. Microscopic examination was used only as a qualitative supporting assessment. Hyphal material was sampled from the pathogen antagonist interaction margin, stained with lactophenol cotton blue, and examined for visible changes in *F. oxysporum* hyphae, including abnormal branching, distortion, and cytoplasmic disintegration. No formal microscopic scoring system was applied, and these observations were not included in the quantitative statistical analysis.

Statistical analysis of the *in vitro* assay

Daily percent inhibition of radial growth (PIRG) was summarized descriptively for each treatment across the seven-day incubation period using the archived treatment means. The experiment comprised two independently conducted runs, with three replicate plates per treatment in each run. The original plate-by-day observations were not available in the revision archive; therefore, day-specific uncertainty estimates and run-adjusted time-course inference was not reconstructed.

Day-7 PIRG was summarized as mean $\pm$ SE with 95% confidence intervals to describe final antagonistic performance. Inhibition-zone widths were similarly summarized as mean $\pm$ SE for the five microbial treatments (T1-T5); inhibition-zone measurements were not applicable to the pathogen-only or carbendazim-treated controls. No inferential tests were applied to the *in vitro* time course, Day-7 PIRG, or inhibition-zone summaries. Statistical inference for the screenhouse phase was conducted as described in the screenhouse trial statistical-analysis section.

Selection of isolates for screenhouse validation

Because T1 and T2 showed the highest and most sustained numerical *in vitro* inhibition of *Fusarium oxysporum* EF1 among the microbial candidates

tested, they were advanced to a screenhouse pot experiment to determine whether their antagonistic activity translated into reduced *Fusarium* wilt development in cotton under controlled plant-associated conditions.

Screenhouse experimental design and inoculation

Cotton (*Gossypium hirsutum* L.) cultivar UKM o8 was used as the host. The screenhouse trial comprised four treatments: (i) an uninoculated healthy control; (ii) a pathogen control inoculated with *Fusarium oxysporum* EF1; (iii) T1 + *F. oxysporum* EF1; and (iv) T2 + *F. oxysporum* EF1. Each treatment comprised 12 individually potted plants, with one plant per pot representing one experimental unit, giving 48 experimental units in total. Pots were randomized among the four treatment groups according to the completely randomized design before screenhouse placement. The exact randomization sequence was not retained in the archived records, and no formal pot-repositioning schedule was documented or is claimed.

Fourteen-day-old UKM o8 seedlings were used for transplantation. The archived experimental records did not retain a verifiable seed source, seed-lot identifier, or independent susceptibility classification for UKM o8; these details are therefore not reconstructed retrospectively. Forest soil classified as sandy loam (pH 6.0-7.0) was steam-sterilized at 121°C and 0.11 MPa for 60 min, cooled, and distributed at 3-5 kg into 3-5-L pots. *Fusarium oxysporum* isolate EF1 was propagated on sterilized rice grains for 10 days, and inoculum density was standardized by propagule counting before incorporation into soil at 10 g pot⁻¹. Infested soil was incubated for 14 days before transplanting to allow establishment and uniform distribution of the pathogen.

Conidial suspensions of T1 and T2 were prepared from actively sporulating cultures, filtered, and adjusted to 1×10^7 conidia mL⁻¹. Immediately before transplanting, seedling roots assigned to

Trichoderma treatments were lightly wounded and immersed in the respective conidial suspension for 10–15 min before transplantation into EF1-infested soil (Harman *et al.*, 2004; Harman, 2006). Root wounding was used to impose a stringent disease-challenge condition and therefore represents greater infection pressure than would normally occur under field conditions. Healthy control plants were grown in non-infested soil without bioagent treatment, whereas pathogen control plants were grown in EF1-infested soil without bioagent treatment.

Plants were maintained for six weeks under ambient screenhouse conditions and irrigated each morning with an equal volume of water per pot; no fertilizer was applied (Fig. 2).



Fig. 2. Representative phenotypic responses of cotton plants at 6 weeks after transplanting under screenhouse conditions

(A) Healthy uninoculated control; (B) T1 + EF1; (C) T2 + EF1; (D) early wilt symptoms; (E) moderate chlorosis and wilting; and (F) severe wilt in the pathogen control. Photographs were taken under comparable conditions using consistent framing where possible. The images are illustrative only; disease severity was evaluated separately using quantitative assessments.

Temperature and relative humidity were not experimentally controlled and should therefore be interpreted as ambient screenhouse conditions. A sterile-rice carrier control was not included. EF1 had been previously characterized and maintained in the TARI Ukiriguru culture collection; although

characteristic wilt symptoms were monitored during the assay, reisolation of EF1 from symptomatic plants was not performed.

Disease assessment and AUDPC

Disease development was assessed weekly for six weeks using a 0–5 *Fusarium* wilt severity scale adapted for cotton: 0 = no symptoms; 1 = one wilted cotyledon; 2 = two wilted or abscised cotyledons; 3 = first true leaf wilted or three leaves abscised; 4 = whole-plant wilting or more than three leaves abscised; and 5 = complete defoliation or plant death (Zhang *et al.*, 2022). Disease incidence was calculated as the percentage of symptomatic plants, and disease severity index (DSI) was calculated from the ordinal scores. Plants that died from wilt were assigned a score of 5 and retained at this score in subsequent assessments. Plants missing for reasons unrelated to disease were excluded from the corresponding assessment denominator. All disease assessments were performed by the same evaluator throughout the six-week experiment to maintain consistency in application of the scoring scale. Blinding of the evaluator to treatment identity was not documented in the experimental records and is therefore not claimed. Disease severity was expressed at the individual-plant level as a normalized severity percentage:

$$\text{Normalized severity (\%)} = (\text{individual plant rating} / 5) \times 100.$$

Treatment-level disease severity index (DSI) was then calculated as the mean normalized severity across all plants within each treatment, equivalent to:

$$\text{DSI (\%)} = [\Sigma (\text{rating} \times \text{number of plants at that rating}) / (5 \times \text{total number of plants assessed})] \times 100.$$

Individual plant normalized severity values, rather than a single aggregated treatment-level DSI, were used as the experimental observations for inferential statistical analyses. Treatment-level DSI values were used to summarize disease severity descriptively.

Cumulative disease development was quantified for each plant as the area under the disease progress curve (AUDPC), calculated by trapezoidal integration of weekly severity scores (Jeger and Viljanen-Rollinson, 2001).

$$\text{AUDPC} = \Sigma [(Y_i + Y_{i+1})/2] \times (t_{i+1} - t_i)$$

AUDPC reduction relative to the pathogen control was calculated as $[(\text{AUDPC control} - \text{AUDPC treatment}) / \text{AUDPC control}] \times 100$.

Statistical analysis of the screenhouse trial

Statistical analyses were performed in R version 4.6.0, with the individual potted plant treated as the experimental unit. Because the screenhouse experiment was conducted once as a single completely randomized design, no run or block term was included. Repeated observations on the same plant were accounted for using plant identity as a random effect where appropriate.

Statistical significance was assessed at $p < 0.05$, and 95% confidence intervals were reported where applicable.

Plant-level area under the disease progress curve (AUDPC), the primary endpoint, was analysed using a linear model with treatment as the fixed effect. Model assumptions were evaluated using residual diagnostics, the Shapiro-Wilk test, Levene's test, and the Breusch-Pagan test. Because heteroscedasticity was detected, treatment effects were evaluated using HC3 heteroscedasticity-robust inference. Prespecified contrasts compared T1 and T2 with the pathogen control, with Holm adjustment for these two comparisons; a direct T1-versus-T2 contrast was also examined. Effect estimates, standard errors, 95% confidence intervals, adjusted P values, and η^2 were reported.

Weekly disease-severity scores were initially analysed using a cumulative-link mixed model with treatment, week, and their interaction as fixed effects and plant identity as a random intercept. Because this ordinal model was numerically unstable, the same repeated-measures structure was analysed using a linear mixed-

effects model as exploratory supportive evidence. Fixed effects were evaluated using *F* tests, and residual diagnostics were used to assess model adequacy; these results were interpreted cautiously because of the ordinal response and departures from normality.

Disease incidence was analysed as a repeated binary outcome using a binomial generalized linear mixed model with a logit link, with treatment, week, and their interaction as fixed effects and plant identity as a random intercept. Global effects were evaluated using Wald χ^2 tests, with model adequacy assessed by simulated-residual diagnostics and checks for sparse-data or separation problems.

At Week 6, overall disease incidence was compared among treatments using Fisher's exact test, followed by prespecified T1-versus-pathogen and T2-versus-pathogen comparisons with Holm adjustment. Plant-level normalized disease severity was analysed using the Kruskal-Wallis test, followed by Holm-adjusted Wilcoxon rank-sum comparisons against the pathogen control. Treatment-level disease severity index values were used descriptively only.

RESULTS

Identification of microbial isolates

Fungal isolates T1 and T2 showed rapid mycelial growth, septate hyphae, branched conidiophores, phialides and abundant green conidia characteristic of *Trichoderma*. Archived BLASTn records indicated 98% similarity between the partial *tef1- α* sequence of T1 and a *Trichoderma harzianum* reference sequence (KC576690), and 99% similarity between T2 and another *T. harzianum* reference sequence (KX254451). These accessions represent reference sequences and were not assigned to the study isolates. The original BLASTn outputs were not retained; therefore, only the archived similarity values are reported. Based on the combined morphological and molecular evidence, T1 and T2 were conservatively classified as putative members of the *T. harzianum* species complex. Assignment to *T. harzianum* sensu stricto was not made because multilocus phylogenetic analysis was not performed.

Species-specific endpoint PCR identified T3 as *Bacillus subtilis* and T4 as *Bacillus amyloliquefaciens*. Identification was supported by amplification of products of the expected sizes in the isolates and positive controls, with no amplification in the no-template controls.

The pathogen EF1, obtained from the TARI Ukiriguru culture collection, had previously been identified as *Fusarium oxysporum* by partial *tef1- α* sequencing, with the sequence deposited in GenBank under accession MK482288.1. Because this partial sequence does not establish host specificity, the isolate is designated *F. oxysporum* EF1 without assignment to forma specialis *vasinfectum*. T1 and T4 originated from Kwimba, T2 from Misungwi and T3 from Magu. These locations indicate isolate provenance only and were not included in the statistical analyses.

In vitro antagonistic activity of microbial isolates

Temporal PIRG profiles showed distinct numerical patterns among the microbial treatments during the seven-day incubation period (Fig. 3). The pathogen-only control remained at 0% PIRG throughout the assay, whereas the carbendazim-treated control represented the 100% inhibition benchmark. Among the microbial treatments, T1 and T2 showed the strongest sustained increases in inhibition. Mean PIRG for T2 increased from 33.3% on Day 1 to 65.9% on Day 7, whereas T1 increased from 38.1% to 61.2% over the same period. The *Bacillus*-based treatments showed lower final inhibition, with Day-7 PIRG values of 36.4% for T4, 34.1% for T3, and 29.5% for the T3+T4 consortium (T5).

The temporal profiles are interpreted descriptively and indicate that T1 and T2 exhibited greater final antagonistic activity than the bacterial treatments under the assay conditions. Because the original plate-by-day dataset required for reproducible run-adjusted repeated-measures inference was not available in the archived analysis materials, no inferential claims regarding treatment-specific temporal slopes or treatment $\times$ time interactions are made.

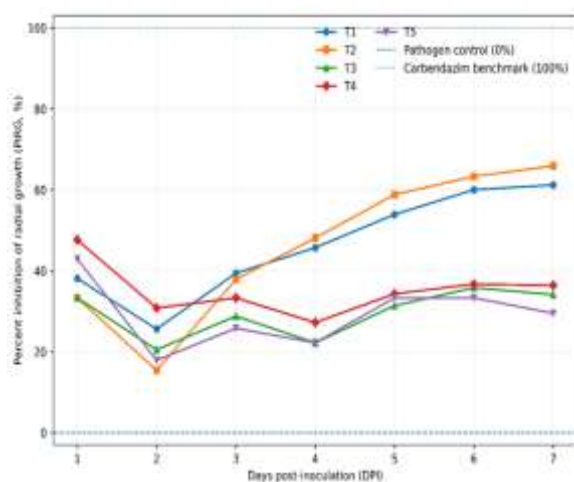


Fig. 3. Temporal changes in percent inhibition of radial growth (PIRG) of *Fusarium oxysporum* EF1 under microbial treatments during seven days of incubation

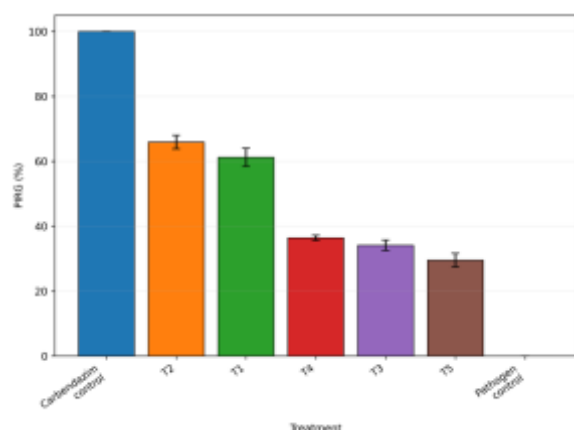


Fig. 4. Day-7 percent inhibition of radial growth (PIRG) of *Fusarium oxysporum* EF1 under microbial treatments and controls. Bars show mean $\pm$ SE ($n = 6$ plates per treatment from two independent experimental runs).

Day-7 PIRG values showed clear numerical differences among the microbial treatments and controls (Fig. 4; Table 2). The carbendazim-treated control completely suppressed growth of *Fusarium oxysporum* EF1, corresponding to 100% PIRG, whereas the pathogen-only control showed 0% inhibition. Among the microbial treatments, T2 produced the highest mean Day-7 PIRG ($65.9 \pm 2.1\%$), followed by T1 ($61.2 \pm 2.8\%$). Lower inhibition was observed for the *Bacillus*-based treatments, with T4, T3, and T5 reaching $36.4 \pm 0.8\%$, $34.1 \pm 1.6\%$, and $29.5 \pm 2.1\%$ PIRG, respectively.

These Day-7 endpoint values are presented descriptively to summarize final antagonistic performance.

Mean inhibition-zone widths were 15.0 ± 4.7 mm for T1, 13.8 ± 2.0 mm for T4, 12.1 ± 2.1 mm for T3, 9.7 ± 2.1 mm for T5, and 9.1 ± 3.5 mm for T2. Considerable variation was observed within treatments, and inhibition-zone measurements produced a numerical ranking that differed from PIRG. The pathogen-only and carbendazim-treated controls were excluded from inhibition-zone summaries because this measurement was not applicable to those treatments.

Microscopic observations

Microscopic examination of the pathogen antagonist interaction zones was performed as a qualitative supporting observation. No clear evidence of mycoparasitic hyphal coiling or penetration of *Fusarium oxysporum* hyphae was observed. Because microscopy was not quantified using a predefined scoring system and representative micrographs were not available for systematic comparison, no mechanistic conclusions were drawn from these observations. The mode of antagonism of the tested bioagents therefore remains unresolved and would require targeted microscopic and biochemical analyses.

Selection of *Trichoderma* isolates for greenhouse validation

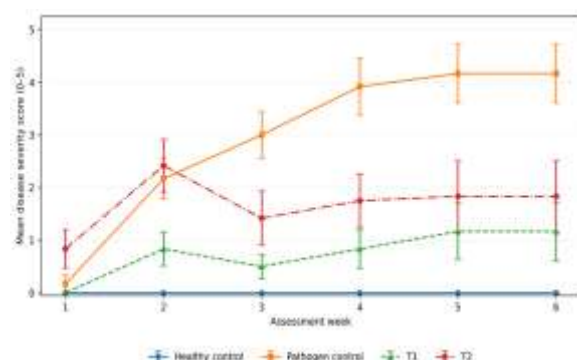
Based on their comparatively strong and sustained numerical inhibition of *Fusarium oxysporum* EF1, the two putative *Trichoderma harzianum* species-complex isolates (T1 and T2) were advanced to a six-week greenhouse pot experiment using the same pathogen isolate (EF1) to determine whether their *in vitro* antagonistic activity translated into suppression of *Fusarium* wilt in cotton.

Screenhouse suppression of *Fusarium* wilt

Fusarium wilt severity diverged progressively among treatments during the six-week screenhouse trial (Fig. 5). The healthy control remained symptom-free throughout, whereas mean severity in the pathogen control increased from 0.17 in Week 1 to 4.17 by Weeks 5–6.

Table 2. Day-7 *in vitro* antagonistic performance of the tested microbial treatments

Treatment	Day-7 PIRG (%) $\pm$ SE	95% CI	Inhibition zone (mm) $\pm$ SE
Carbendazim control	100.0 $\pm$ 0.0	100.0-100.0	Not applicable
T2	65.9 $\pm$ 2.1	60.5-71.3	9.1 $\pm$ 3.5
T1	61.2 $\pm$ 2.8	54.0-68.4	15.0 $\pm$ 4.7
T4	36.4 $\pm$ 0.8	34.3-38.5	13.8 $\pm$ 2.0
T3	34.1 $\pm$ 1.6	30.0-38.2	12.1 $\pm$ 2.1
T5	29.5 $\pm$ 2.1	24.1-34.9	9.7 $\pm$ 2.1
Pathogen control	0.0 $\pm$ 0.0	0.0-0.0	Not applicable

**Fig. 5.** Weekly progression of *Fusarium* wilt severity in cotton under screenhouse conditions (0-5 scale)

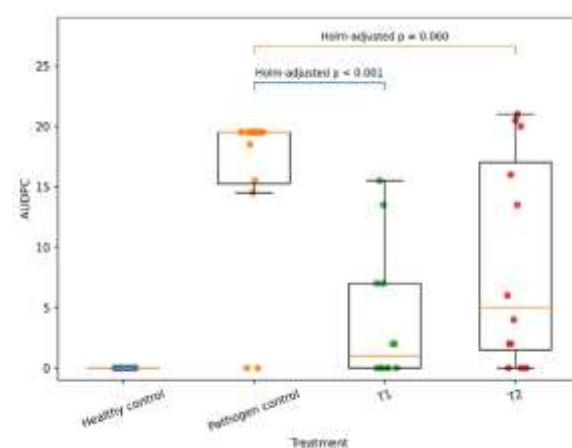
Points show treatment means and error bars show $\pm$ SE. The pathogen control showed progressive disease increase, whereas T1 maintained the lowest mean severity, followed by T2.

Among pathogen-inoculated plants, T1 consistently showed the lowest disease severity, reaching a mean score of 1.17 at Week 6, compared with 1.83 for T2. Because the cumulative-link ordinal mixed model was numerically unstable, weekly severity scores were analysed using a linear mixed-effects model as exploratory supportive evidence. The model showed significant effects of treatment ($F(3,48) = 13.64$, $p < 0.001$) and week ($F(1,240) = 88.91$, $p < 0.001$), together with a significant treatment $\times$ week interaction ($F(3,240) = 36.27$, $p < 0.001$), indicating that disease progression differed among treatments over time. Model residuals deviated from normality (Shapiro–Wilk $W = 0.944$, $p < 0.001$); therefore, these LMM results were interpreted as supportive rather than confirmatory evidence.

Cumulative disease development (AUDPC)

Cumulative disease development differed among treatments (Fig. 6). The pathogen control had the highest mean AUDPC (15.42 ± 2.14), followed by T2

(8.75 ± 2.52) and T1 (3.92 ± 1.62), whereas the healthy control remained disease-free (0.00 ± 0.00). Relative to the pathogen control, mean AUDPC was reduced numerically by 74.6% with T1 and 43.2% with T2.

**Fig. 6.** Cumulative *Fusarium* wilt development expressed as area under the disease progress curve (AUDPC) during the six-week screenhouse trial

Because heteroscedasticity was detected (Levene's $F = 4.00$, $p = 0.013$; Breusch–Pagan = 9.76, $p = 0.021$), HC3 heteroscedasticity-robust inference was used for the primary AUDPC analysis. The overall treatment effect was significant, $F(3,44) = 21.38$, $p = 1.08 \times 10^{-8}$.

Prespecified robust contrasts showed that T1 had significantly lower AUDPC than the pathogen control (estimate = -11.50 AUDPC units, SE = 2.80, 95% CI: -17.14 to -5.86 , Holm-adjusted $p < 0.001$). In contrast, the numerical reduction observed for T2 did not reach statistical significance under robust inference (estimate = -6.67 , SE = 3.45, 95% CI: -13.63 to 0.29 , Holm-adjusted $p = 0.060$). The direct robust comparison between T1 and T2 was also not

significant (estimate = -4.83, SE = 3.13, 95% CI: -11.14 to 1.47, $p = 0.130$). Thus, T1 showed the larger numerical reduction in cumulative disease, but the two isolates were not statistically distinguished.

Final disease status at Week 6

At Week 6, the healthy control remained disease-free (0/12 symptomatic plants), whereas the pathogen control had 10/12 symptomatic plants (83.3% incidence) and a mean normalized severity of $83.3 \pm 11.2\%$. T1 had 4/12 symptomatic plants (33.3% incidence; normalized severity $23.3 \pm 11.2\%$), while T2 had 5/12 symptomatic plants (41.7% incidence; normalized severity $36.7 \pm 13.4\%$) (Fig. 7). Normalized severity was calculated for each plant as (severity score/5) $\times$ 100, and treatment values are presented as mean $\pm$ SE of the individual

plant-level percentages. Overall Week-6 incidence differed among treatments using an exact 4×2 Fisher test ($p < 0.001$). Prespecified pairwise Fisher exact comparisons of each *Trichoderma* treatment against the pathogen control were adjusted using the Holm procedure and did not reach statistical significance (T1: adjusted $p = 0.072$; T2: adjusted $p = 0.089$); these results indicate uncertainty in the incidence differences rather than evidence of equivalence. In contrast, Week-6 normalized disease severity differed among treatments (Kruskal-Wallis $\chi^2(3) = 20.02$, $p < 0.001$), with significantly lower severity for both T1 (Holm-adjusted $p = 0.007$) and T2 (Holm-adjusted $p = 0.010$) relative to the pathogen control. Individual plant-level severity values and their distributions are shown in Fig. 7.

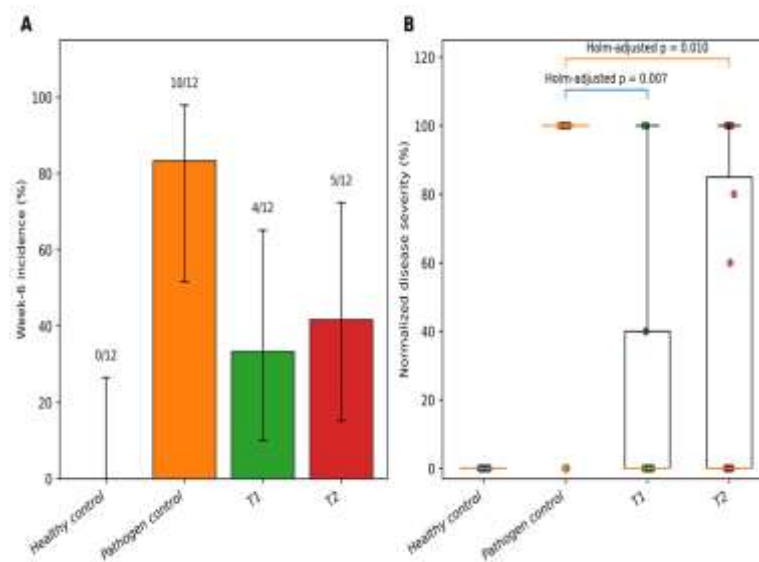


Fig. 7. Week-6 *Fusarium* wilt incidence and disease severity across treatments

(A) Percentage of symptomatic plants at Week 6; numbers above bars indicate symptomatic plants/total plants assessed ($n = 12$), and error bars represent 95% exact binomial confidence intervals. (B) Distribution of plant-level normalized disease severity percentages at Week 6, calculated as (severity score/5) $\times$ 100. Points represent individual plants, and boxplots show the treatment distributions. Brackets indicate Holm-adjusted Wilcoxon comparisons of T1 and T2 with the pathogen control; adjusted p values are shown.

Comparison of *in vitro* and greenhouse performance

A descriptive comparison of the two assay systems revealed a change in the numerical ranking of T1 and T2. In the dual-culture assay, T2 produced slightly greater Day-7 inhibition than T1 (65.9% vs. 61.2% PIRG) (Table 3). In the greenhouse trial, T1 showed a greater numerical reduction in AUDPC

relative to the pathogen control than T2 (74.6% vs. 43.2%). However, the direct robust comparison of greenhouse AUDPC between T1 and T2 was not statistically significant ($P = 0.130$). Thus, the observed difference in ranking should be interpreted as numerical rather than evidence of statistically established superiority of either isolate.

Table 3. Summary of the principal analyses used to evaluate *in vitro* antagonism and screenhouse disease suppression

Endpoint	Analysis / contrast	Estimate (95% CI)	SE	(df)	Multiplicity adjustment	p-value
Seven-day PIRG time course (<i>in vitro</i>)	Descriptive treatment-by-day summary across two experimental runs	Mean PIRG by treatment and day	-	NA	NA	NA
Day-7 PIRG (<i>in vitro</i>)	Descriptive endpoint summary	Mean $\pm$ SE and 95% CI	-	NA	NA	NA
Inhibition-zone width (<i>in vitro</i>)	Descriptive summary of T1-T5	Mean $\pm$ SE	-	NA	NA	NA
Weekly disease severity	Exploratory repeated-measures LMM: treatment	-	-	F (3,48) = 13.64	None	1.46×10^{-6}
Weekly disease severity	Exploratory repeated-measures LMM: week	-	-	F (1,240) = 88.91	None	3.68×10^{-16}
Weekly disease severity	Exploratory repeated-measures LMM: treatment $\times$ week	-	-	F (3,240) = 36.27	None	2.30×10^{-19}
AUDPC	HC3	$\eta^2 = 0.471$ (95% CI: 0.270-1.000)	-	F (3,44) = 21.38	None	1.08×10^{-8}
AUDPC	T1 vs pathogen control,	-11.50 (-17.14 to -5.86)	2.80	t (44) = -4.11	Holm	<0.001
AUDPC	T2 vs pathogen control,	-6.67 (-13.63 to 0.29)	3.45	t (44) = -1.93	Holm	0.060
AUDPC	T1 vs T2	-4.83 (-11.14 to 1.47)	3.13	t (44) = -1.54	None; prespecified	0.130
Repeated disease incidence	Binomial GLMM: treatment $\times$ week	-	-	χ^2 (3) = 10.004	None	0.0185
Week-6 incidence	Overall, 4 $\times$ 2	-	-	Exact test	None	2.18×10^{-4}
Week-6 incidence	T1 vs pathogen control,	-	-	Exact test	Holm	0.072
Week-6 incidence	T2 vs pathogen control, Fisher exact test	-	-	Exact test	Holm	0.089
Week-6 normalized disease severity	Overall Kruskal-Wallis test	-	-	χ^2 (3) = 20.018	None	1.68×10^{-4}
Week-6 normalized disease severity	T1 vs pathogen control, Wilcoxon rank-sum test	Not estimated	-	Rank-based test	Holm	0.0068
Week-6 normalized disease severity	T2 vs pathogen control, Wilcoxon rank-sum test	Not estimated	-	Rank-based test	Holm	0.0103

DISCUSSION

Biocontrol activity of indigenous *Trichoderma* isolates

The principal finding was that the two indigenous *Trichoderma* isolates performed more strongly than the *Bacillus*-based treatments *in vitro* and suppressed *Fusarium* wilt development when evaluated in cotton plants. T1 and T2 produced 61.2% and 65.9% Day-7 PIRG, respectively, and both significantly reduced Week-6 normalized disease severity relative to the pathogen control. T1 also significantly reduced cumulative disease development as measured by AUDPC, whereas the adjusted T2-versus-pathogen contrast was marginal but not significant. These results identify both isolates as promising candidates for further evaluation, while providing stronger statistical support for T1 under the specific screenhouse conditions used here. This interpretation is consistent with reports that *Trichoderma* spp. can suppress soil-borne pathogens through multiple, strain-dependent traits (Harman *et al.*, 2004; Woo *et al.*, 2023). It should nevertheless be restricted to controlled conditions because neither field efficacy nor local adaptation was tested.

Potential mechanisms of antagonism

The sustained increase in PIRG produced by T1 and T2 is compatible with several mechanisms described for *Trichoderma*, including rapid resource competition, secretion of antifungal metabolites and hydrolytic enzymes, and direct interactions with pathogen hyphae (Harman *et al.*, 2004; Vinale *et al.*, 2008; Marzano *et al.*, 2013). The qualitative hyphal alterations observed at the colony interface support the presence of an antagonistic interaction, but they do not distinguish antibiosis, enzyme-mediated lysis, competition, or mycoparasitism. Because metabolite production, enzyme activity, and direct parasitism were not quantified, the present data demonstrate inhibition rather than a particular mechanism.

Bacillus subtilis T3 and *B. amyloliquefaciens* T4 produced moderate inhibition of *F. oxysporum* EF1. Strains within these species can produce antifungal lipopeptides such as iturins, fengycins, and surfactins (Kim *et al.*, 2010), but those compounds were not measured in T3 or T4. Moreover, lipopeptide profiles can vary with nutritional and rhizosphere conditions (Nihorimbere *et al.*, 2012), so activity on PDA should not be assumed to represent activity around cotton

roots. The T3+T4 consortium inhibited the pathogen less than either component alone, suggesting that combining strains did not confer an additive benefit under the assay conditions. Whether this response reflected competition, antagonism, or another compatibility constraint remains unknown and should be tested before any multi-strain formulation is developed.

Relationship between *in vitro* and screenhouse performance

The screenhouse experiment extended the agar assay by incorporating the host and soil environment, although the sterilized substrate and wounded-root inoculation procedure remained artificial. Across the six-week period, AUDPC was numerically lower with both isolates than with the pathogen control. The HC3 robust planned contrast confirmed a reduction for T1, whereas the Holm-adjusted contrast for T2 did not meet the 0.05 threshold ($p = 0.060$). AUDPC is particularly informative here because it integrates disease intensity over time rather than describing a single assessment (Jeger and Viljanen-Rollinson, 2001; Madden *et al.*, 2007). The exploratory repeated-measures analysis and the endpoint analyses therefore converge on delayed or reduced disease development, but the strength of evidence was not identical for the two isolates.

The assays also produced different numerical rankings. T2 had slightly higher Day-7 PIRG than T1 (65.9% versus 61.2%), whereas T1 produced the larger reduction in AUDPC relative to the pathogen control (74.6% versus 43.2%). Neither result establishes that one isolate was superior: the Day-7 comparison was descriptive, and the direct T1-versus-T2 AUDPC contrast was not significant ($p = 0.130$). This ranking shift is biologically plausible because agar assays mainly reflect direct antagonism, whereas plant-based performance may also depend on root colonization, rhizosphere competence, persistence, metabolite expression, and host defence induction (Druzhinina *et al.*, 2011; Mukherjee *et al.*, 2013; Woo *et al.*, 2023). Those traits were not measured here, so they are hypotheses for follow-up rather than

explanations demonstrated by the present study. The observed shift nevertheless shows why sequential *in vitro* and host-based screening is more informative than selection from agar inhibition alone.

Disease incidence and severity conveyed complementary information. Although both *Trichoderma* treatments had lower numerical Week-6 incidence than the pathogen control, their pairwise incidence contrasts were not significant after Holm adjustment. In contrast, both isolates significantly reduced Week-6 normalized severity. Incidence records whether symptoms are present, whereas severity reflects their intensity (Campbell and Madden, 1990; Madden *et al.*, 2007). Thus, under the imposed disease challenge, the treatments limited symptom development more clearly than they prevented symptom onset. This distinction is important when judging practical value: a candidate that slows disease may still be useful in an integrated programme, but it should not be described as providing complete protection.

Implications for *Fusarium* wilt management in cotton

Recovery of T1 and T2 from cotton rhizosphere soils makes them relevant candidates for local development, but origin alone does not demonstrate adaptation, persistence, or superiority in farmers' fields. The results support advancing both isolates to more realistic validation stages, with T1 prioritized for its stronger AUDPC evidence and T2 retained because it reduced endpoint severity and showed the highest numerical inhibition *in vitro*. Neither isolate is ready for deployment on the basis of this study alone.

Study limitations and future research

Several limitations define the scope of inference. First, both stages were conducted under controlled conditions. Field soil heterogeneity, temperature and moisture variation, resident microbial communities, pathogen pressure, and crop management can all alter biocontrol performance (Fravel *et al.*, 2003). The screenhouse assay further used steam-sterilized sandy-loam soil and lightly wounded roots to create a

consistent disease challenge. These choices strengthened experimental control but limit extrapolation to non-sterile soil and natural infection.

Second, the screenhouse experiment included one *F. oxysporum* isolate, one cotton cultivar, and 12 plants per treatment. The findings therefore cannot establish consistency across pathogen populations, host genotypes, locations, or seasons. A sterile-rice carrier control and *Trichoderma*-only treatments were not included, and the pathogen was not reisolated from symptomatic plants at the end of the trial. These omissions restrict attribution of carrier effects, assessment of bioagent effects in the absence of disease, and confirmation of the causal organism in individual symptomatic plants.

Third, only T1 and T2 were advanced to the screenhouse. Selection largely on agar-based antagonism may favor traits expressed on artificial medium and exclude isolates with weaker *in vitro* inhibition but stronger rhizosphere competence. Because the *Bacillus* treatments were not tested in plants, the study supports comparisons among all candidates only for the *in vitro* phase; it provides no evidence about the screenhouse or field efficacy of T3, T4, or T5.

Fourth, the study did not quantify colonization, persistence, hydrolytic-enzyme activity, antifungal metabolites, or host defence responses. Microscopy was qualitative and cannot establish mycoparasitism or another specific mechanism. Taxonomic resolution was also limited: T1 and T2 were assigned conservatively to the putative *T. harzianum* species complex from morphology and a partial *tef1-alpha* sequence, without study-specific GenBank accessions or multilocus phylogenetic analysis.

Finally, disease severity was scored on an ordinal scale. Because the planned ordinal mixed model was numerically unstable, the linear mixed model was treated as exploratory support; AUDPC and the Week-6 non-parametric analysis provided the principal severity inferences. This analytical distinction should be retained when interpreting the reported p values.

Future work should test both isolates in non-sterile soil and under natural infection across multiple cotton cultivars, *F. oxysporum* isolates, locations, and growing seasons. Such studies should quantify root colonization and persistence, confirm taxonomic identity using multilocus evidence, and examine enzyme activity, antifungal metabolites, and host responses. Formulation stability, dose, delivery method, compatibility with agronomic practices, and effects on non-target organisms also require evaluation before field use is considered (Lorito *et al.*, 2010; Yao *et al.*, 2023). For consortia, explicit compatibility testing should precede combined application.

CONCLUSION

This study identified two indigenous *Trichoderma* isolates with promising antagonistic activity against *Fusarium oxysporum* EF1. Both isolates produced substantial numerical inhibition *in vitro* and significantly reduced Week-6 normalized disease severity under screenhouse conditions. T1 produced the larger numerical reduction in cumulative disease, although the direct AUDPC comparison between T1 and T2 was not statistically significant.

The differing numerical rankings between the *in vitro* and screenhouse assays indicate that agar-based antagonism alone may not predict relative performance under plant-associated conditions. Both isolates therefore warrant further validation under non-sterile soil and field conditions, including assessment of persistence, colonization, formulation, application methods, and consistency across cotton cultivars and pathogen isolates.

ACKNOWLEDGEMENTS

The authors thank the Nelson Mandela African Institution of Science and Technology (NM-AIST) for academic support, and the Tanzania Agricultural Research Institute (TARI) for financial support, research facilities and technical assistance. We also thank the field and laboratory staff who facilitated sample collection, laboratory analysis and the screenhouse trial.

REFERENCES

- Aslam S, Baber M, Naqqash T, Javed M, Bredenbruch S, Grundler FMW, Schleker ASS.** 2025. Rhizospheric *Bacillus* isolates control Fusarium wilt on cotton and enhance plant biomass and root development. *Frontiers in Microbiology* **16**, 1580937.
DOI: 10.3389/fmicb.2025.1580937
- Barnett HL, Hunter BB.** 1998. Illustrated genera of imperfect fungi. 4th ed. APS Press, St. Paul, Minnesota, USA.
- Bell AA, Kemerait RC, Ortiz CS, Prom S, Quintana J, Nichols RL, Liu J.** 2017. Genetic diversity, virulence, and *Meloidogyne incognita* interactions of *Fusarium oxysporum* isolates causing cotton wilt in Georgia. *Plant Disease* **101(6)**, 948–956.
DOI: 10.1094/PDIS-09-16-1382-RE
- Campbell CL, Madden LV.** 1990. Introduction to plant disease epidemiology. Wiley, New York, USA.
- Carbone I, Kohn LM.** 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91(3)**, 553–556.
DOI: 10.1080/00275514.1999.12061051
- Cianchetta AN, Davis RM.** 2015. *Fusarium* wilt of cotton: Management strategies. *Crop Protection* **73**, 40–44.
DOI: 10.1016/j.cropro.2015.01.014
- Davis RM, Colyer PD, Rothrock CS, Kochman JK.** 2006. Fusarium wilt of cotton: Population diversity and implications for management. *Plant Disease* **90(6)**, 692–703.
DOI: 10.1094/PD-90-0692
- Dennis C, Webster J.** 1971. Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society* **57(1)**, 25–39.
DOI: 10.1016/S0007-1536(71)80077-3
- Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP.** 2011. *Trichoderma*: The genomics of opportunistic success. *Nature Reviews Microbiology* **9(10)**, 749–759.
DOI: 10.1038/nrmicro2637
- Duan Y, Chen R, Zhang R, Jiang W, Chen X, Yin C, Mao Z.** 2021. Isolation, identification, and antibacterial mechanisms of *Bacillus amyloliquefaciens* QSB-6 and its effect on plant roots. *Frontiers in Microbiology* **12**, 746799.
DOI: 10.3389/fmicb.2021.746799
- Esmail S, Oelbermann M.** 2022. Investigating farmer perspectives and compost application for soil management in urban agriculture in Mwanza, Tanzania. *Frontiers in Soil Science* **2**, 905664.
DOI: 10.3389/fsoil.2022.905664
- Fravel D, Olivain C, Alabouvette C.** 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist* **157(3)**, 493–502.
DOI: 10.1046/j.1469-8137.2003.00700.x
- Harman GE, Howell CR, Viterbo A, Chet I, Lorito M.** 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology* **2(1)**, 43–56.
DOI: 10.1038/nrmicro797
- Harman GE.** 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* **96(2)**, 190–194.
DOI: 10.1094/PHYTO-96-0190
- Huang C-H, Huang L, Chang M-T, Wu C-P.** 2017. Use of novel specific primers targeted to *pheS* and *tuf* genes for species and subspecies identification and differentiation of *Bacillus subtilis* subsp. *subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus licheniformis*. *African Journal of Microbiology Research* **11(7)**, 264–270.
DOI: 10.5897/AJMR2017.8434

- Javed I, Khan MA, Riaz H, Saeed S, Sajid M, Bashair M, Abbas Z, Ali H, Al-Dosary MA, Junaid MB.** 2025. Biological approaches to sustainable management of Fusarium wilt in cotton. *Journal of Crop Health* **77**, Article 69. DOI: 10.1007/s10343-025-01137-5
- Jeger MJ, Viljanen-Rollinson SLH.** 2001. The use of the area under the disease progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theoretical and Applied Genetics* **102(1)**, 32–40. DOI: 10.1007/s001220051615
- Kim PI, Ryu J, Kim YH, Chi YT.** 2010. Production of biosurfactant lipopeptides iturin A, fengycin, and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. *Journal of Microbiology and Biotechnology* **20(1)**, 138–145. DOI: 10.4014/jmb.0905.05007
- Kwon GH, Lee HA, Park JY, Kim JS, Lim J, Park CS, Kwon DY, Kim YS, Kim JH.** 2009. Development of a RAPD-PCR method for identification of *Bacillus* species isolated from Cheonggukjang. *International Journal of Food Microbiology* **129(3)**, 282–287. DOI: 10.1016/j.ijfoodmicro.2008.12.013
- Logan NA, De Vos P.** 2015. *Bacillus*. In: Whitman WB (ed.). *Bergey's manual of systematics of Archaea and Bacteria*. Wiley, 1–163. DOI: 10.1002/9781118960608.gbm00530
- Lorito M, Woo SL, Harman GE, Monte E.** 2010. Translational research on *Trichoderma*: From 'omics to the field. *Annual Review of Phytopathology* **48**, 395–417. DOI: 10.1146/annurev-phyto-073009-114314
- Madden LV, Hughes G, van den Bosch F.** 2007. *The study of plant disease epidemics*. APS Press, St. Paul, Minnesota, USA. DOI: 10.1094/9780890545058
- Marzano M, Gallo A, Altomare C.** 2013. Improvement of biocontrol efficacy of *Trichoderma harzianum* vs. *Fusarium oxysporum* f. sp. *lycopersici* through UV-induced tolerance to fusaric acid. *Biological Control* **67(3)**, 397–408. DOI: 10.1016/j.biocontrol.2013.09.008
- Mfinanga F, Panga B, Temba J.** 2025. Enhancing cotton yield through adoption of climate-smart agriculture: A case of smallholder farmers in Bariadi District, Tanzania. *Turkish Journal of Agriculture - Food Science and Technology* **13(S3)**, 3776–3784. DOI: 10.24925/turjaf.v13is3.3776-3784.7993
- Michielse CB, Rep M.** 2009. Pathogen profile update: *Fusarium oxysporum*. *Molecular Plant Pathology* **10(3)**, 311–324. DOI: 10.1111/j.1364-3703.2009.00538.x
- Mukherjee PK, Horwitz BA, Herrera-Estrella A, Schmoll M, Kenerley CM.** 2013. *Trichoderma* research in the genome era. *Annual Review of Phytopathology* **51**, 105–129. DOI: 10.1146/annurev-phyto-082712-102353
- Ndole RW, Kilasi NL, Hamisi MI, Saidia PS.** 2023. Screening of selected cotton genotypes (*Gossypium* spp.) for resistance to Fusarium wilt disease in Tanzania. *Journal of Current Opinion in Crop Science* **4(4)**, 172–178. DOI: 10.62773/jcocs.v4i4.215
- Nihorimbere V, Cawoy H, Seyer A, Brunelle A, Thonart P, Ongena M.** 2012. Impact of rhizosphere factors on cyclic lipopeptide signature from the plant beneficial strain *Bacillus amyloliquefaciens* S499. *FEMS Microbiology Ecology* **79(1)**, 176–191. DOI: 10.1111/j.1574-6941.2011.01208.x
- Sambrook J, Russell DW.** 2001. *Molecular cloning: A laboratory manual*. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sanogo S, Zhang J. 2016. Resistance sources, resistance screening techniques and disease management for Fusarium wilt in cotton. *Euphytica* **207(2)**, 255–271.

DOI: 10.1007/s10681-015-1532-y

Skidmore AM, Dickinson CH. 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Transactions of the British Mycological Society* **66(1)**, 57–64.

DOI: 10.1016/S0007-1536(76)80092-7

Tanzania Cotton Board. 2026. MHE. Chongolo: Uhakika wa pembejeo, matumizi ya teknolojia kuleta tija ya zao la pamba. Retrieved from <https://www.tcb.go.tz/news/hon-chongolo-reliable-access-to-inputs-and-use-of-technology-to-drive-cotton-productivity>

Tarazi R, Santos-Jiménez JL, Vaslin MFS. 2020. Biotechnological solutions for major cotton (*Gossypium hirsutum*) pathogens and pests. *Biotechnology Research and Innovation* **3**, 19–26.

DOI: 10.1016/j.biori.2020.01.001

United Republic of Tanzania. 2017. Climate-smart agriculture guideline for the United Republic of Tanzania: A country-driven response to climate change, food and nutrition insecurity. Food and Agriculture Organization of the United Nations, Rome, Italy. Retrieved from <https://www.fao.org/3/i7157e/i7157e.pdf>

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. 2008. *Trichoderma*-plant-pathogen interactions. *Soil Biology and Biochemistry* **40(1)**, 1–10.

DOI: 10.1016/j.soilbio.2007.07.002

Woo SL, Hermosa R, Lorito M, Monte E. 2023. *Trichoderma*: A multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. *Nature Reviews Microbiology* **21(5)**, 312–326.

DOI: 10.1038/s41579-022-00819-5

Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J. 2023. *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology* **14**, 1160551.

DOI: 10.3389/fmicb.2023.1160551

Zhang J, Abdelraheem A, Zhu Y, Elkins-Arce H, Dever J, Whitelock D, Hake K, Wedegaertner T, Wheeler TA. 2022. Studies of evaluation methods for resistance to Fusarium wilt race 4 (*Fusarium oxysporum* f. sp. *vasinfectum*) in cotton: Effects of cultivar, planting date, and inoculum density on disease progression. *Frontiers in Plant Science* **13**, 900131.

DOI: 10.3389/fpls.2022.900131

Zhou Y, Zhang H, Martin TK, Chagoya J, Isakeit T, Shim WB. 2026. Pathogenicity and virulence of *Fusarium oxysporum* f. sp. *vasinfectum* race 4: Understanding an emerging threat to cotton from field to genome. *Virulence* **17(1)**, 2682654.

DOI: 10.1080/21505594.2026.2682654