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Morphology, Diagnostics, and Biological Significance of Trichoderma afroharzianum Identified in the Agroecosystems of Uzbekistan

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Abstract. In this study, the biodiversity of microfungi belonging to the genus Trichoderma, isolated from diverse agroecological regions of Uzbekistan, was comprehensively analyzed using integrated mycological and taxonomic approaches. During the investigation, the morpho-cultural characteristics, vegetative growth kinetics, and microscopic parameters of the sporulation apparatus (including conidiophore architecture, phialide morphometry, and conidial ultrastructure) were determined for species identification. For the first time, the

morphological markers of

indigenous *T. afroharzianum* strains were systematized, establishing their significant role in enriching the "Phytopathogenic and Other Microorganisms" unique collection at the Institute of Genetics and Plant Experimental Biology, Academy of Sciences of the Republic of Uzbekistan. The findings serve as a fundamental basis for evaluating the mycoparasitic and antagonistic potential of these fungi, as well as for selecting source cultures for the development of high-efficiency biotechnological preparations aimed at protecting agricultural crops from phytopathogens.

Keywords: *Trichoderma afroharzianum*, identification, morphometry, conidiophore, biodiversity, phytopathogenic fungi, biocontrol, mycoparasitism.

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O‘zbekiston agroekotizimlarida aniqlangan Trichoderma afroharzianum mikomitsetining morfologiyasi, diagnostikasi va biologik ahamiyati

Annotatsiya Ushbu tadqiqotda O‘zbekistonning turli agroekologik mintaqalaridan ajratib olingan Trichoderma turkumiga mansub mikomitsetlarning bioxilma-xilligi kompleks mikologik

va taksonomik yondashuvlar yordamida har tomonlama tahlil qilindi. Tadqiqot davomida turlarni identifikatsiya qilish uchun morfo-madaniy xususiyatlar, vegetativ o‘sish kinetikasi va sporulyatsiya apparatining mikroskopik ko‘rsatkichlari (jumladan, konidioforlar arxitekturasi, fialidlar morfometriyasi va konidiyalarning ultrastrukturasi) aniqlandi. Ilk bor mahalliy T. afroharzianum shtammlarining morfologik markerlari tizimlashtirildi va ularning O‘zbekiston Respublikasi Fanlar akademiyasi Genetika va o‘simliklar eksperimental biologiyasi instituti qoshidagi “Fitopatogen va boshqa mikroorganizmlar” noyob ob’ektlar kolleksiyasini boyitishdagi muhim roli belgilandi. Olingan natijalar ushbu zamburug‘larning mikoparazitar va antagonistik salohiyatini baholash, shuningdek, qishloq xo‘jaligi ekinlarini fitopatogenlardan himoya qilishga qaratilgan yuqori samarali biotexnologik preparatlarni ishlab chiqishda boshlang‘ich kulturalarni tanlash uchun fundamental asos bo‘lib xizmat qiladi.

Kalit so‘zlar: Trichoderma afroharzianum, identifikatsiya, morfometriya, konidiofor, fialid, bioxilma-xillik, fitopatogen zamburug‘lar, biokontrol, mikoparazitizm.

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Морфология, диагностика и биологическое значение Trichoderma afroharzianum, идентифицированного в агроэкосистемах Узбекистана

Аннотация В данном исследовании с использованием комплексных микологических и таксономических подходов проведен всесторонний анализ биоразнообразия микромицетов рода Trichoderma, выделенных из различных агроэкологических регионов Узбекистана. В ходе работы для идентификации видов были определены морфолого-культурные признаки, кинетика вегетативного роста и микроскопические параметры споруляционного аппарата (включая архитектуру конидиеносцев, морфометрию фиалид и ультраструктуру конидий). Впервые систематизированы морфологические маркеры аборигенных штаммов T. afroharzianum, что обосновывает их важную роль в пополнении уникальной коллекции «Фитопатогенные и другие микроорганизмы» при Институте генетики и экспериментальной биологии растений Академии наук Республики Узбекистан. Полученные результаты служат фундаментальной основой для оценки микопаразитического и антагонистического потенциала данных грибов, а также для отбора исходных культур при разработке высокоэффективных биотехнологических препаратов, направленных на защиту сельскохозяйственных культур от фитопатогенов.

Ключевые слова: Trichoderma afroharzianum, идентификация, морфометрия, конидиеносец, фиалида, биоразнообразие, фитопатогенные грибы, биоконтроль, микопаразитизм.

Introduction

Soil ecosystems represent the most complex microbial habitats on Earth, harboring diverse populations of bacteria, actinomycetes, and fungi. The microbial density in suppressive soils can reach several billion cells per gram of dry substrate, with an overall biomass ranging from 1 to 8 tons per hectare [1]. Within this microbiota, phytopathogenic fungi pose a significant threat to global food security, inducing root rot, vascular wilts, and necrotic diseases that severely compromise crop yields. Historically, agricultural systems have heavily relied on synthetic fungicides and fumigants; however, their excessive application has led to environmental degradation, soil toxicity, and the emergence of resistant pathogen strains [4]. Consequently, there

is an urgent paradigm shift toward sustainable biocontrol agents (BCAs) that can mitigate diseases while enhancing plant systemic resistance.

Among the various BCAs, the genus *Trichoderma* (Ascomycota, Hypocreales) stands out as a preeminent group of filamentous fungi widely utilized in both agriculture and forestry. Their multifaceted efficacy is governed by complex mechanisms, including antibiosis via the synthesis of secondary metabolites, and mycoparasitism facilitated by the secretion of cell wall-degrading enzymes (CWDEs) such as chitinases and β-1,3-glucanases [5, 6]. *Trichoderma harzianum* and the recently reclassified *T. afroharzianum* are recognized as exceptionally potent antagonists. Recent genomic studies have highlighted the significance of the Epl-1 (Eliciting plant response-like 1) gene family, which encodes cerato-platanin proteins that function as elicitors, triggering plant immune responses and modulating pathogen-host interactions [2, 7].

Trichoderma species are prolific producers of over a hundred antimicrobial secondary metabolites, including trichomycin, viridin, trichodermin, gliotoxin, and various peptabiols [3, 8]. These bioactive molecules not only inhibit the growth of soil-borne pathogens like *Fusarium oxysporum* and *Rhizoctonia solani* but also function as plant growth promoters (PGP). Despite the global documentation of these fungi, the specific morphological characteristics and biocontrol potential of indigenous *T. afroharzianum* strains in the unique semi-arid agroecosystems of Uzbekistan remain insufficiently characterized.

Given the escalating challenges of climate change and soil salinity in Central Asia, identifying locally adapted *Trichoderma* lineages is critical for developing region-specific biopesticides. This study aims to characterize the morphology and diagnostic features of *T. afroharzianum* isolates from Uzbekistan and evaluate their biological significance as a foundational step for sustainable biotechnological applications.

Materials and methods

Study Area and Soil Sampling Soil samples were collected from diverse agroecosystems in Uzbekistan, specifically from the Shafirkan district (Bukhara region), Yunusobod district Botanika garden, and Qibray district. To evaluate soil health and microbial fertility, samples were obtained from two distinct coordinates within each site at depths of 10, 20, and 30 cm using sterile containers.

Isolation of Antagonistic Fungi The isolation of *Trichoderma* species was performed using the modified soil dilution method. Briefly, 10 g of each soil sample was suspended in 300 ml of sterile distilled water. Serial dilutions were prepared at ratios of 10^{-2}, 10^{-3}, and 10^{-4}. Aliquots of 1 ml from each dilution were aseptically inoculated onto Potato Dextrose Agar (PDA) plates. All inoculation procedures were conducted under a laminar flow cabinet to ensure aseptic conditions

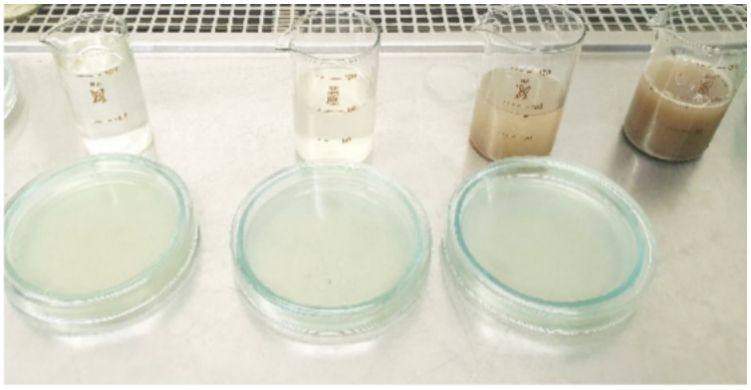

Figure 1. Preparation of soil suspensions and inoculation onto PDA medium using the modified soil dilution method.

Cultivation and Purification Inoculated plates were incubated at 26±2 C for 72 hours. Emerging fungal colonies were subjected to mycological examination and purified using the mono-spore isolation technique until stable pure cultures were obtained.

Result and discussion

Morphological Characterization and Identification The identification of the isolated fungal strains was conducted according to the *"Morphology and Identification of Trichoderma"* taxonomic keys. The isolates demonstrated rapid radial growth on PDA medium, initially forming white aerial mycelium that subsequently transitioned to characteristic green or light-green shades upon sporulation (Fig. 2).

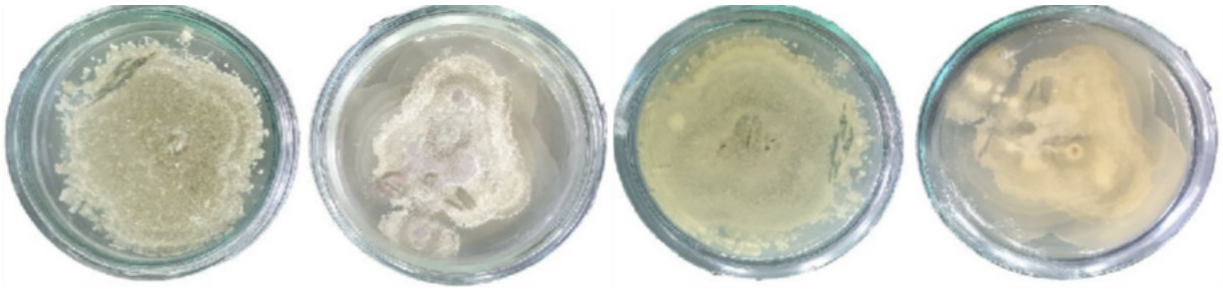

Figure 2. Pure culture of *Trichoderma afroharzianum* on PDA medium after 72 hours of incubation: (a) obverse view, (b) reverse view.

Microscopic Analysis Microscopic observation of the GPEB-65 isolate (Fig. 3) revealed specific diagnostic features of *Trichoderma afroharzianum*. The mycelium consists of multinucleated hyphae that serve as the structural framework for conidiogenesis. The conidia were found to be globose to sub-globose (oval) in shape, organized in clusters or short chains at the termini of divergent phialides. The branching pattern of the conidiophores and the specific morphometry of the conidia confirm the taxonomic identity of the strain as *T. afroharzianum* [2, 4].

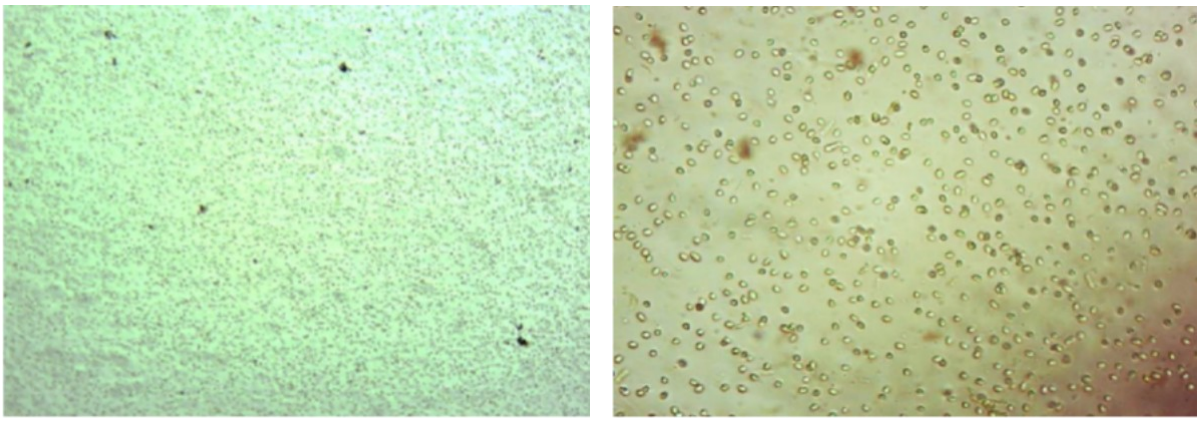

Figure 3. Micromorphology of *T. afroharzianum* isolate: branched conidiophores and oval-shaped conidia in clusters (Magnification: 40x/100x).

Conclusion

Morphological and microscopic analyses of *Trichoderma* isolates obtained from diverse agro-ecological regions of Uzbekistan successfully determined their taxonomic composition. The study identified the species *Trichoderma afroharzianum*, which exhibits high adaptability and widespread distribution within the country's specific soil-climatic conditions. The growth kinetics, colony pigmentation, and micromorphological parameters of conidiogenesis indicate that this species occupies a distinct ecological niche within regional agroecosystems.

The findings provide a scientific basis for evaluating the biodiversity of indigenous *Trichoderma* strains and highlight their potential as effective antagonists against aggressive phytopathogenic fungi. Consequently, the development and integration of locally-sourced biopreparations based on *T. afroharzianum* into agricultural practice hold strategic importance for reducing reliance on synthetic fungicides. Future research should focus on evaluating the antagonistic efficiency of these strains under field conditions across various crops, including cotton, wheat, and vegetables.

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