OPEN ACCESS Int. J. Phytopathol. 14 (02) 2025. 139-149 DOI: 10.33687/phytopath.014.02.5531

Check for updates

Available Online at EScience Press

International Journal of Phytopathology

ISSN: 2312-9344 (Online), 2313-1241 (Print)

https://esciencepress.net/journals/phytopath

FIRST REPORT OF PEPPER DAMPING-OFF CAUSED BY *FUSARIUM OXYSPORUM* IN UZBEKISTAN

[a]Dilrabo T. Aznabakieva, [b]Batyr A. Khasanov, [c]Anvar G. Sherimbetov, [c]Bakhtiyor Sh. Adilov, [d]Dilshod R. Ruzmetov

[a]Department of Plant Protection, Institute of Agriculture and Agri-Technologies, Andijan 170600, Uzbekistan.

[b]Department of Plant Pathology, Tashkent State Agrarian University, Tashkent 100140, Uzbekistan.

[c]Laboratory of Plant Immunity, Institute of Genetics and Plant Experimental Biology, Academy of Sciences of Uzbekistan, Tashkent Region 111226, Uzbekistan.

[c] Collection of Plant Pathogenic and Other Microorganisms, Institute of Genetics and Plant Experimental Biology, Academy of Sciences of Uzbekistan, Tashkent Region 111226, Uzbekistan.

ARTICLE INFO

Article History
Received: February 26, 2025
Revised: May 29, 2025
Accepted: August 28, 2025

Keywords
Capsicum annuum
Damping-off
Fusarium oxysporum
Morphological identification
Pathogenicity
Molecular identification
TEF-1α

ABSTRACT

Sweet and hot peppers (*Capsicum annuum* L.), widely cultivated vegetable and spice cultures worldwide are attacked by various pathogenic fungi and oomycetes. Among them *Fusarium* species, most often *F. oxysporum*, cause various diseases, including the damping-off. Fusarium diseases on *Capsicum* spp. and other vegetable crops were also reported in Uzbekistan, but pathogens were identified only morphologically, based on characteristics of their colonies and sporulation organs. However, it is currently required that morphological identification must necessarily be accompanied by molecular genetic identification and the fulfillment of Koch's postulates. The present study was conducted in order to identify damping-off pathogens of sweet pepper seedlings in Uzbekistan caused by species of the genus *Fusarium*, considering these requirements. In 2022, the incidence of seedling damping-off in a greenhouse nursery in the Andijan region of Uzbekistan was 12.5%. In the mycological analysis, *Fusarium*-like colonies grew out of 58% of the symptomatic plant parts. Two representative isolates, UZF-21 and UZF-23 have been morphologically identified as *Fusarium oxysporum*. Molecular genetic analysis of the partial sequences of the *TEF*-1α gene of these isolates and BLAST search have confirmed the results of morphological identification. The received sequences were deposited in the NCBI database under GenBank accession numbers OQ466308 and OQ466309, respectively. This is the first report of *F. oxysporum* as the cause of bell pepper damping-off, which identity was confirmed in Uzbekistan using both morphological and molecular analyses.

Corresponding Author: Batyr A. Khasanov
Email: khasanov.batyr@gmail.com

INTRODUCTION

Both sweet or bell pepper and hot or chili pepper widely cultivated in many countries of the world mostly belong to the single species *Capsicum annuum* L. Pepper fruits occupy an important place in people's diets worldwide, including Uzbekistan. In 2021, the global production of sweet pepper was 34.6 million tons, with China accounting for 17.6 M tons, or 51 percent of the world production. Other production leaders were Mexico and Turkey that produced 2.3 and 2.2 M tons, respectively (Munene, 2022). There are no exact data on amounts of sweet pepper fruits produced in Uzbekistan, but reports

say that their export is increasing in the country, and it has exceeded 13,900 tons in 2021 (Romakaeva, 2021).

In 2022, 4,909,320 tons of dry chili pepper was harvested in total worldwide. Top producers were India that produced 1,874,000 tons (38,17% of the total volume) followed by Bangladesh with 624,825 tons (12,73%). Uzbekistan stood 22nd with 22,673 tons of production (FAOSTAT, 2019).

Pepper diseases are poorly studied in Uzbekistan. It is only indicated (Khasanov *et al*., 2021) that certain fungi cause seedling blight and root rot of adult plants (*Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* Kühn), stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), fruit rot (*Botrytis cinerea* Pers., *Alternaria* spp., *Fusarium* spp.), powdery mildew (*Leveillula taurica* (Lév.) G. Arnaud) and vascular wilts (*Fusarium* spp., *Verticillium* spp.). All these species were identified morphologically, but no molecular identification methods were applied.

Sustainable production of high pepper yields is threatened by various abiotic and biotic stresses, including diseases. Review of the available literature data has shown that >58 species of fungi and >11 species of fungus-like organisms (oomycetes *Pythium* and *Phytophthora*) can cause pepper diseases worldwide (Khasanov *et al*., 2021). The most serious diseases of pepper include Fusarium wilt, Fusarium root, crown, stem and fruit rots, powdery mildew, anthracnose, Phytophthora blight, and some others (e. g., Tsitsigiannis *et al*., 2018; Gabrekiristos *et al*., 2020a). Some of these organisms cause pepper damping-off as well. This paper will focus on pepper damping-off caused by *Fusarium* spp. that are considered a group of the most common and important causal agents of this disease.

Damping-off is an emerging disease worldwide, which affects all agricultural and other crops, both in nurseries and fields. It can be a problem in high-tunnel and greenhouse production systems. There is no country or geographic area without damping-off problems, on a number of economically important crops (Lamichhane *et al*., 2017).

Damping-off is the first disease that vegetable growers are most likely to observe during the growing season (Melanson, 2022). It can affect numerous hosts, including peppers, tomatoes, eggplants, cucurbits, and can be a threat for vegetable production both in greenhouses and outdoors. Damping-off symptoms can be observed from seeding until the fourth to sixth week post-sowing (Horst, 2013). Seedlings are susceptible to damping-off from pre-germination to the time when first true leaves are produced, then plants become less susceptible (Grabowski, 2018).

There are two types of the disease – pre-emergence damping-off and post-emergence damping-off. Pre-emergence damping-off affects seeds before or after germination, and seedlings before emergence from the soil. Post-emergence damping-off is rotting and collapse of seedlings soon after they emerge from the soil (Horst, 2013). Plants beyond the seedling stage that are affected by damping-off pathogens are typically said to be affected by crown, root, and/or stem rots rather than damping-off (Grabowski, 2018; Melanson, 2022). Damping-off is often favored by prolonged cool, damp growing conditions that slow germination and seedling growth, extending the time seedlings are most susceptible, excess nitrogen in growing media, and overcrowding of seedlings (Grabowski, 2018; Melanson, 2022).

Pre-emergence damping-off can make seeds soft, mushy, with gray-brown discoloration of the radicle, hypocotyl, and cotyledons, and unable to germinate. Hyphae may be present on the affected tissues. Water-soaked lesions can be formed on rootlets and hypocotyls, that may turn reddish-brown or black, increase in size, girdle young stems resulting in wilting and death of seedlings before emergence. As a result, bare spaces can be formed in the seedling stand (Horst, 2013).

Post-emergence damping-off symptoms are seedling decay, wilting, and death after emergence.

Common symptoms include development of water-soaked, then necrotic sunken lesion at the lower stem, usually at or below the soil line, causing the plant to fall over, collapse and die. This type of the disease is most common in warm humid weather (Horst 2013; Lamichhane *et al*., 2017, and references therein).

Damping-off is caused by many semi-parasitic true fungi and oomycetes, that can live in soil as saprophytes. Over 30 species of fungi and oomycetes that belong to more than 15 genera are reported as a cause of the damping-off but only a few of them are frequently associated with the disease (Horst 2013; Lamichhane *et al*., 2017). The most common pathogens that cause damping-off in vegetables and other field crops are fungi, *Rhizoctonia* spp. and *Fusarium* spp., and oomycetes, *Pythium* spp. and *Phytophthora* spp. (Lamichhane *et al*., 2017, and references therein; Grabowski, 2018; Meadows *et al*., 2020; Melanson, 2022). Some authors consider that species of the genera *Botrytis, Sclerotinia, Thielaviopsis,*

Macrophomina, Cylindrocladium, Diplodia and *Sclerotium* may be important on occasion (Horst 2013).

In general, species of *Pythium* and *Phytophthora* often occur under cold and wet conditions and in low-lying areas that have poor drainage and hold water, and often attack plant parts below the soil surface. *Fusarium* and *Rhizoctonia* species occur under warmer and drier conditions, and often attack seedlings at the soil line and cause post-emergence damping-off (Meadows *et al*., 2020; Melanson, 2022). Several species from these genera can occur on host plants simultaneously. Species of *Fusarium*, *Rhizoctonia* and *Phytophthora* may infect also crowns, roots and fruits of older plants.

Damping-off pathogens tend to have wide host ranges and are common in fields as soil inhabitants or persisting in the form of survival or reproductive structures (chlamydospores of *Fusarium* spp., sclerotia of *Rhizoctonia* spp., etc.), or by colonizing crop debris and other organic matter. Disease occurrence and severity depend on the amount of inoculum and environmental conditions present in the field (Melanson, 2022).

Propagules of the damping-off pathogens can be introduced into the seedling tray with pots, tools, and potting media that have been used previously and harbor the pathogens. Garden soil used as a potting media also may contain propagules of the pathogens. Pathogens can be spread by insects, rain drops, splashing water, and worker's hands and clothing. The damping-off pathogens may be spread within fields in infested soil particles, rain drops, and contaminated equipment (e. g., Grabowski, 2018).

Once introduced to a seedling tray, the damping-off pathogens easily move from plant to plant by growing through the potting media. Damping-off often first infects just a few plants but can spread quickly throughout the entire tray and into neighboring flats. Seedlings infected with damping-off often are killed. If the problem is extensive, replanting of fields or transplant trays may be required (Grabowski, 2018).

True damping-off may be confused with symptoms of some abiotic stresses including soil heat, soil crusting, excessive wetness / dryness of soil, improper use of fertilizers and chemicals, phytotoxicity of certain pesticides, etc. Such symptoms can be distinguished from those caused by biotic stress because the damage from abiotic issues is generally scattered throughout nurseries/seedbeds at or near 100% incidence, while that caused by biotic stresses often occurs in expanding patches (Lamichhane *et al*., 2017, and references therein; Meadows *et al*., 2020).

The control of damping-off diseases in vegetables requires an integrated disease management approach combining sanitation, cultural, chemical, biological, and other non-chemical methods. The ways of using these methods and definite control means are quite well covered in the literature (Horst 2013; Gholve *et al*., 2014; Lamichhane *et al*., 2017; Grabowski, 2018; Tsitsigiannis *et al*., 2018; Collins, 2020; Meadows *et al*., 2020; Melanson, 2022).

In Uzbekistan pepper growers that produce seedlings in their own nurseries often face the damping-off problem. The post-emergence damping-off has been found in each of nurseries of two districts of Andijan region where bell pepper seedlings were grown. The purpose of our research was to determine the incidence of this disease, isolate its causal agent(s) in a pure culture, and identify them by morphological and molecular characteristics, as well as to fulfill Koch's postulates with them.

MATERIALS AND METHODS

Sample Collection, Isolation and Morphological Characteristics of Causal Agents

In 2021 and 2022 the damping-off of sweet pepper (*Capsicum annuum*) seedlings has occurred in several commercial greenhouses nurseries of the Andijan Region, Uzbekistan. To determine the pathogen and disease incidence, the nursery of the "Universal Agro-Luks" private farm in the Shakhrikhon District has been selected as a model object. In this nursery, farmers grew seedlings of the sweet pepper cv. Ferrari F_1 on the area of 172.9 m^2. To identify pathogens 25 died or dying (fallen over) cotyledons and/or young seedlings at one to two true leaf growth stage were collected and brought to the Plant Immunity laboratory, Institute of Genetics and Plant Experimental Biology (IGPEB, Kibray District, Tashkent Region), Academy of Sciences of Uzbekistan. Five to 10 mm sections from each sample were cut with a sterile blade, surface sterilized with 1% NaOCl for 30 s and 75% ethanol for 30 s, rinsed thrice with sterile distilled water, air dried between two layers of the sterile filter paper in the Laminar flow box, and transferred to potato dextrose agar (PDA) amended with 50 μg/ml of penicillin G and streptomycin sulphate to exclude any bacterial growth. Petri dishes were incubated in the thermostat at 25°C in the dark and observed periodically. After four days hyphal

tips from emerged *Fusarium*-like colonies were transferred to PDA and carnation leaf agar (CLA) media, and eight single-spore cultures were obtained. From these isolates, two were randomly selected for morphological and molecular genetic identification. These representative isolates were identified morphologically following sporulation. Pure cultures of these two isolates were deposited in the Collection of Phytopathogenic Microorganisms of the IGPEB (accession nos. UZF-21 and UZF-23).

The subcultures of these two isolates were grown for 10-15 days on PDA and CLA media for morphological identification. Culture characteristics of each isolate, and microscopic features of conidia and conidiophores were determined based on Leslie and Summerell (2006).

Molecular Characterization

To verify morphological identification, genomic DNA was extracted from two representative isolates UZF-21 and UZF-23 using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, USA). DNA concentration was estimated using spectrophotometer NanoDrop Eight (Thermo Fisher Scientific, USA). DNA samples were stored at –20°C until further use.

For molecular genetic identification of *Fusarium* species *TEF*-1α gene was chosen because the scientific experts in a field of fungal molecular genetic identification recommend using this gene that presents as a single-copy gene in the *Fusarium* genus and demonstrates a high level of sequence polymorphism among the closely related *Fusarium* species, compared with other DNA markers such as ITS (internal transcribed spacer - noncoding spacer DNA located between the SSU and LSU rRNAs), SSU 18S rDNA (small subunit ribosomal DNA), LSU 28S rDNA (large subunit ribosomal DNA), TUBB (β-tubulin), calmodulin and etc. Therefore, *TEF*-1α alone is able to differentiate all species of this genus and has become the preferred marker as a single-locus detection tool in *Fusarium* (O'Donnell *et al*., 2015; O'Donnell *et al*., 2022).

A fragment of the translation elongation factor-1 alpha (*TEF*-1α) gene from these strains was amplified using primers EF1 and EF2 designed by O'Donnell *et al*. (2015, 2022). Amplification reactions were conducted using Platinum hot start PCR 2x master mix (Thermo Fisher Scientific, USA) in a total volume of 25 µl containing 12,5 µl 2X Master Mix, 1 µl of each primer (10 µM), and 4 µl of the cDNA. 4 µl of genomic DNA and 3,5 µl DNA ddH2O. The PCRs were performed using a MiniAmp Plus Thermal Cycler (Thermo Fisher Scientific, USA). The conditions were an initial denaturation at 94°C for 2 min; followed by 40 cycles of 94°C for 30 s, 57°C for 45 s, 72°C for 1 min, and a final extension at 72°C for 5 min.

Agarose gel electrophoresis (2%, stained with ethidium bromide (EtBr) at a final concentration of 0.5 µg/ml) was used to detect amplified PCR products of *TEF*-1α. Electrophoresis was performed at 100 V and 80 mA for 100 min with 1X TBE buffer (pH 8.3) using horizontal electrophoresis system SE-1 (Helicon, Russia). The obtained bands were evaluated using 100 bp DNA Ladder (Thermo Fisher Scientific, USA). After electrophoresis, the gel was visualized under ultraviolet (UV) light using a gel document imaging system BK-AG100 (Biobase Kings Co., Ltd, China).

PCR products purified using the PureLink™ Quick Gel Extraction Kit (Thermo Fisher Scientific, USA) according to manufacturer's instructions.

DNA sequencing was performed in the Laboratory of Plant Immunity at the IGPEB using the BigDye® Terminator v 3.1 kit (Thermo Fisher Scientific, USA). Cycle sequencing reaction consists of ddH_2O- 3.5 µl, BigDye- 1 µL, 5x Seg.buffer-2 µl, sequencing primer - 0.5 µl, purified PCR product - 2 µl. EF1 and EF2 primers were used for sequencing. The conditions of cycle sequencing reaction were initial denaturation at 96°C for 1 minute; followed by 45 cycles of 96°C for 10 s, 57°C for 10 s, 60°C for 3 min. The sequencing reaction products were purified from fluorescently labeled terminator nucleotides using the Dynabeads Sequencing Clean-Up Kit (Thermo Fisher Scientific, USA). Sequencing was conducted on an ABI 3500 DNA sequencer (Thermo Fisher Scientific, USA).

The *TEF*-1α nucleotide sequences for each isolate were then compared to those in the public domain databases NCBI (National Center for Biotechnology Information; www.ncbi.nih.gov) using Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN). Multiple sequence alignments of *TEF*-1α sequences were conducted using ClustalW and Muscle. Maximum likelihood phylogenetic trees were generated using MEGA 11 (Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al*., 2021). Bootstrapping test (with 1000 bootstrap replications) was used to estimate the confidence of the branches in a phylogenetic tree.

Pathogenicity Tests

The pathogenicity of two isolates, UZF-21 (Treatment 1) and UZF-23 (Treatment 2), to the bell pepper cv. Ferrari F_1

was tested using modified "sickpot" method (Pandey *et al*. 2020) in December 2022 to February 2023. The inoculum used was a mycelial-spore suspension, 10^9 conidia/ml, prepared by grinding the mycelium of 14-day old cultures in a blender. The substrate was a mixture of garden soil and sand (3:1) sterilized at 70°C for 60 minutes. 100 ml of inoculum of each isolate was separately mixed with 1 kg of the substrate and incubated for 7 days at room temperature for the colonization of fungus. Plastic pots with a diameter of 3.8 cm were used in this test. The pots were preliminarily disinfested by placing them into 10% solution of the sodium hypochlorite for 2 min, then washed thoroughly with sterile water and air-dried. Approximately 400 grams of the inoculated soil mixture was transferred to each of pots. To accelerate germination, pepper seeds were moistened with sterile distilled water and incubated in a thermostat at 29±1°C for 72 hours. In each sickpot 10 seeds were sown to a depth of ~ 2.5 cm. One sickpot with 10 sown seeds was considered to be one replication. The pots were arranged in a randomized complete block design in three replications in the glasshouse conditions. In the healthy check Treatment 3 the seeds were sown in the pots with a substrate containing no inoculum. All pots were kept in a greenhouse for 30 days from the date of seeds sown, with an additional illumination (500 klx), at a temperature of 25±2°C during the day (16 h) and 19±1°C at night (8 h). The pots were watered as required. After 30 days of incubation, the seedlings from each pot were carefully dug out separately, washed with water and examined for symptoms of the disease. Disease assessment was performed by counting the number of seedlings emerged and died, measuring their height, and examining the hypocotyls for other symptoms.

RESULTS

Sample Collection, Isolation and Morphological Characteristics of Causal Agents

In 2022, the damping-off of sweet pepper (*Capsicum annuum*, cv. Ferrari F_1) seedlings has occurred on the area of 172.9 m^2 in the commercial greenhouse nursery of the "Universal Agro-Luks" private farm. The incidence of the post-emergence damping-off was determined by counting the total number of seedlings at the flat and the number of dead and dying ones at 8 randomly chosen points 0.5 m^2 each. In average, there were 47.9 seedlings per 0.5 m^2, and 6.0 (12.5%) of them were fallen over and dying (Figure 1).

For mycological analysis 25 symptomatic seedlings were collected, and two fragments from infected lower parts of each seedling were cut and sown on Petry dishes with PDA medium (5 fragments per plate) as stated in the "Materials and Methods" section. Twenty nine, or 58% of all segments in the analysis yielded uniform on morphology colonies with whitish mycelium resembling that of *Fusarium* fungi.

Two representative isolates of the fungi designated as UZF-21 and UZF-23 were fast growing and diameter of their colonies on the PDA medium has reached 50 to 60 mm in five days. Colonies were white initially and turned pale-yellow or yellowish-pale-red in the center of some cultures (Figure 2).

Figure 1. Dead or dying sweet pepper seedlings in the greenhouse nursery.

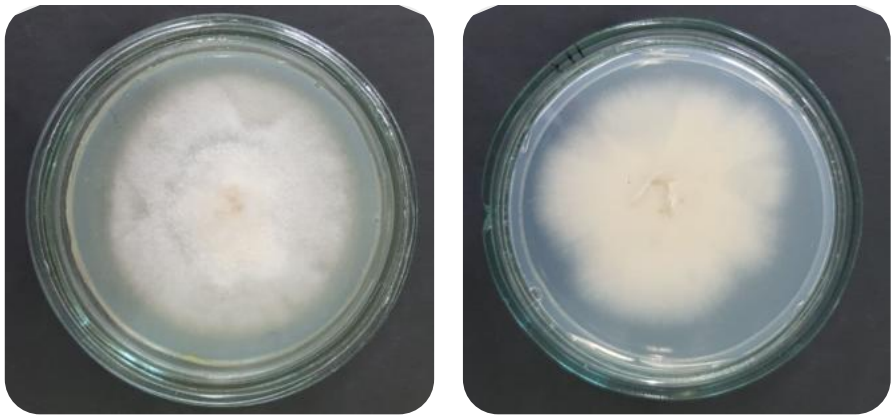

Figure 2. Surface (left) and reverse (right) view of the *Fusarium oxysporum* UZF-23 strain colony after 9 days growth on PDA at 23 C.

For studying microscopic characteristics, the isolates were grown on PDA and CLA media. Sporodochial macroconidia (n=50) of the both isolates were mostly with three septa, straight to slightly curved, and measured 22.5 to 29.5 x 2.6 to 5.2 µm. Microconidia were born on the short monophialides, abundant, non-septate, ovoid-elliptical shaped, and measured 5.2 to 12.8 x 2.2 to 3.8 µm (Figure 3). Chlamydospores on PDA medium were terminal and intercalary, single or in pairs, non-septate, and measured 6.7 to 10.6 µm in diameter. These features of conidia were typical for *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen (Leslie and Summerell, 2006; Lombard *et al*. 2019).

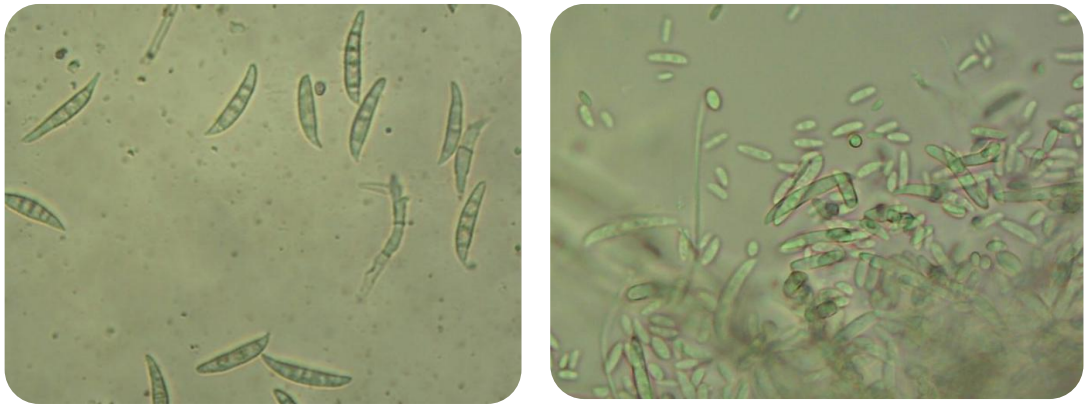

Figure 3. Macronidia and microconidia of *Fusarium oxysporum* UZF-21 strain on PDA. ~400X.

Molecular Identification

To verify morphological identification, DNA was extracted from isolates UZF-21 and UZF-23, and a portion of the translation elongation factor-1 alpha (*TEF*-1α) gene from these strains was amplified using primers EF1 and EF2 (O'Donnell *et al*. 2022), and amplicons obtained were sequenced. The *TEF*-1α sequences of two isolates, UZF-21 and UZF-23 were deposited in GenBank with accession nos. OQ466308 and OQ466309, respectively. BLAST analysis with the FUSARIUM-ID databases and NCBI found that *TEF*-1α of UZF-21 (GenBank: OQ466308) was 100% identical to that of reference sequences of *Fusarium oxysporum* GenBank accession number OP487223 (*Fusarium oxysporum* f. sp. *cubense* E. F. Sm., Snyder & Hansen, tropical race 4, synonym: *Fusarium odoratissimum* Maryani *et al*.) and MZ359496 (*Fusarium oxysporum* strain isolated from a keratitis patient), and 99.81% to that of accession numbers MN417204, MK266474, MH093663, and KT239473, isolated from sesame, ginseng, pennycress, and onion, respectively. *TEF*-1α of UZF-23 (GenBank: OQ466309) was 99.74% identical to that of *Fusarium oxysporum* GenBank accession numbers MN329692, MN450387, MK752475, and LC514570, isolated from soil, vanilla, potato, and

plumbing drain, respectively (Table 1). A maximum likelihood phylogenetic tree was created in MEGA11 based on *TEF*-1α sequences. *TEF*-1α similarity of OQ466308 and OQ466309 with *Fusarium oxysporum* strains recovered from the symptomatic bell pepper crowns and/or roots elsewhere (Perez- Hernández *et al*. 2014; Lomas-Cano *et al*. 2014; Velarde-Félix S. *et al*. 2018) (GenBank accession nos.KF928951, HG916993, and KT780441, respectively) was 96.83% and 98.45%, respectively (Table 1).

Pathogenicity Tests

The germination rate of the pepper seeds in all treatments was 100%, and they emerged in 4-5 days after sowing. All pepper seedlings grown in six sickpots (Treatment 1 and Treatment 2) inoculated with UZF-21 and UZF-23 have shown signs of infection. The main symptom of the disease was stunting – the height of all seedlings was 2-2.5 times less than that of the control plants (10 to14 cm against 20 to 24 cm, respectively). Some of infected seedlings have fallen over and died after emergence on the soil surface. After 30 days of incubation, 3 to 5 infected seedlings have died in each of Treatment 1 and Treatment 2, and the number of dead plants were 10 to 15% on average. The affected seedlings retained their green color, but the lower part of the hypocotyls thinned, and some seedlings had light brownish spots on them. All control plants in the Treatment 3 remained healthy. The same pathogens used for inoculation were successfully reisolated from the symptomatic and dead seedlings onto PDA, thus fulfilling Koch's postulates with both isolates (UZF-21 and UZF-23).

Table 1. Results of BLAST analysis for *TEF*-1α gene sequences of *Fusarium oxysporum* strains isolated from bell pepper seedlings.

Strain name	Species name	NCBI GenBank accession number	DNA target	BLAST match sequence		
				Reference accession №	Coverage (%)	Identity (%)
UZF-21	*Fusarium oxysporum*	OQ466308	*TEF*-1α	OP487223	100%	100%
				MZ359496	100%	100%
				MN417204	100%	99.81%
				MK266474	100%	99.81%
				MH093663	100%	99.81%
				KT239473	100%	99.81%
UZF-23	*Fusarium oxysporum*	OQ466309	*TEF*-1α	MN329692	100%	99.74%
				MN450387	100%	99.74%
				MK752475	100%	99.74%
				LC514570	100%	99.74%

DISCUSSION

Fusarium oxysporum has been identified as the causal agent of the damping-off disease on pepper seedlings in the greenhouse nursery in the Andijan region, Republic of Uzbekistan. This pathogen has been identified using both morphological and molecular methods for the first time in the country. These results may provide information for the implementation of disease control measures for Fusarium diseases during production of pepper seedlings in the greenhouses.

The damping-off of various agricultural and forest crops has long been a well-known disease in nurseries, and since the 2000s, its prevalence has been increasing in different countries of the world. It has been shown that >30 species of fungi and oomycetes can cause this disease, but the most common of them were species of the genera *Fusarium*, *Rhizoctonia*, *Pythium,* and *Phytophthora* (Horst 2013; Lamichhane *et al*., 2017). Among more than a dozen species of the genus *Fusarium* listed in the literature as pathogens of pepper (Lamichhane *et al*., 2017; López-Orona *et al*., 2019; Khasanov *et al*., 2021),

Fusarium oxysporum probably dominates in terms of occurrence in this culture. Various populations of this species or its forma speciales, in addition to damping-off, cause various diseases on sweet and hot pepper plants (and other nightshade crops), including wilt, root rot, crown rot, stem rot and/or fruit rot; these diseases can occur in nurseries, greenhouses, and in open fields throughout the growing season (Sanogo, 2003; Velásquez-Valle *et al*., 2007; Lomas-Cano *et al*., 2016;

Lamichhane *et al*., 2017). So, *F. oxysporum* has caused economically important pepper diseases in nurseries and greenhouses in Spain (Lomas-Cano *et al*., 2014; Pérez-Hernández *et al*., 2014), Italy (Gilardi *et al*., 2019), USA (Mmbaga *et al*., 2018), and Canada (Cerkauskas, 2017). Another destructive form of the disease is Fusarium wilt, which occurs in the world wherever pepper is cultivated. Wilt is one of the major diseases of hot pepper in India (e.g., Khan *et al*., 2018; Pandita *et al*., 2019), Ethiopia, the USA and Egypt (e.g., Gabrekiristos *et al*., 2020a,b,c, and references therein; Oljira, Berta, 2020).

Although Fusarium diseases of various crops are intensely studied in the world, identification of the pathogens to the level of species and forma spp. is still challenging (Leslie & Summerell, 2006). Thus, some call the causal agent of pepper wilt simply *F. oxysporum* (Khan *et al*., 2018; Pandita *et al*., 2019; Oljira, Berta, 2020; Shaheen *et al*., 2021; Engalycheva *et al*. 2024), while others name it *F. oxysporum* f. sp. *capsici* Rivelli (Sanogo, 2003; Velarde-Félix *et al*., 2018; Altinok *et al*., 2020; Gabrekiristos *et al*., 2020a,b,c; El-kazzaz *et al*., 2022). The taxonomic legitimacy of the f. sp. *capsici* is still unsolved. Based on the complete coincidence of the *TEF*-1α sequences of the two isolates, Lomas-Cano *et al*. (2016) concluded that the name *F. oxysporum* f. sp. *capsici* is synonymous to *F. oxysporum* f. sp. *radicis-capsici*.

A group of the Mexican scientists have come to even more surprising conclusions (López-Orona *et al*., 2019). In their experiments, they used 10 isolates that were previously received from pepper plants and identified by Velarde-Félix *et al*. (2018) as *F. oxysporum* f. sp. *capsici*. Based on analyses of *TEF*-1α and some other genes, as well as studies of their pathogenicity to pepper, tomato, eggplant and 7 other crops, they concluded that these 10 isolates are conspecific with the pathogen of tomato wilt, *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen, race 2 (López-Orona *et al*., 2019). All 10 isolates of the pathogen in these experiments affected all 9 species of test hosts (López-Orona *et al*., 2019). At the same time, in the cross-inoculation experiments of other researchers (Sanogo, 2003; Cerkauskas, 2017; Altinok *et al*., 2020) *F. oxysporum* isolates from pepper plants affected only pepper, but not tomato and eggplant; *F. oxysporum* f. sp. *radicis-capsici* also did not infect tomato plants (Lomas-Cano *et al*., 2016). These and other literature data indicate the need for further research on the identification of *Fusarium oxysporum* and its special forms affecting pepper and other vegetable crops.

The results of the current study have shown that the main causal agent of damping-off disease in greenhouse nurseries in Andijan Region of Uzbekistan was *Fusarium oxysporum*, which has been isolated from 58% of symptomatic pepper seedling segments. The other causal agent of the disease was *Rhizoctonia solani* Kühn, which was isolated from 10% of the symptomatic seedling segments (data not shown).

Global experience shows that four groups of Integrated Disease Management (IDM) methods should be applied against damping-off disease of pepper, namely: (i) pre-sowing seed treatment; (ii) growing resistant and/or tolerant cultivars; (iii) application of the best cultural techniques and (iv) application of chemical and/or biological fungicides. However, none of these groups is effective if applied individually, therefore, all of them should be applied as a complex (Lamichhane *et al*., 2017). At the same time, measures against damping-off in peppers and other vegetable crops in Uzbekistan have not been studied, and local farmers use only some elements of agronomic and sanitary-hygienic methods of control. There is also no fungicide registered in the country (as seed treatment, soil drench, or spray) against this disease. Therefore, scientists and specialists in the field of plant protection in Uzbekistan face the following tasks: study of pepper seedling diseases in other regions of the country and identification of their causal agents; screening of currently cultivated and newly introduced from other countries pepper varieties for resistance/tolerance to damping-off caused by *F. oxysporum* and other fungal species; studying under the conditions of Uzbekistan the effectiveness of chemical and biological fungicides that have shown high efficiency against damping-off pathogens in other countries.

It should be noted that in order to choose the appropriate control tactics against pepper damping-off disease (for example, choosing the appropriate fungicide), it is important to identify properly the pathogen that is causing the disease (Lamichhane *et al*., 2017; Melanson, 2022). While studying morphological characteristics is important, it is not sufficient for correct identification of the pathogen species. Therefore, morphological identification must always be confirmed by molecular analysis, which involves determining the DNA base sequences of informative genes. In Uzbekistan, the molecular method is still used to a limited extent for identifying plant pathogenic fungi, and in this work, it was

used for the first time to identify *F. oxysporum* as the causative agent of damping-off disease in pepper seedlings. Undoubtedly, studies of plant pathogenic fungi, based on both classical morphological methods and DNA analysis of informative genes, will be used more widely in the coming years, ensuring their reliable identification in Uzbekistan.

CONCLUSION

Cultural, microscopic, and molecular analyses as well as the pathogenicity test revealed that *Fusarium oxysporum* was the main causal agent for pepper damping-off disease in two greenhouses in Uzbekistan. To our knowledge, this is the first report of *F. oxysporum* as the cause of bell pepper damping-off, which identity was confirmed in the country for the first time using molecular analysis of the *TEF*-1α.

In the future, it is necessary to expand research in this area in the country, monitor the occurrence of damping-off disease on pepper plants (and other vegetable crops), and determine the composition of its pathogens in other regions of the country, as well as to search for varieties of these crops that are less susceptible or are tolerant to this disease. It is also necessary to conduct research in order to introduce sanitary, chemical, and biological methods of control against diseases of pepper seedlings and other vegetable crops into local plant protection practices.

Knowledge on the genetic diversity of *F. oxysporum* can be utilized to manage the damping-off and root rot diseases, improve plant stands, and help pepper breeding programs in Uzbekistan.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in publishing the article.

AUTHORS CONTRIBUTIONS

Prof. Batyr Khasanov developed this idea, facilitated, guided, and supervised the experiment, and wrote an initial draft. Mrs. Dilrabo Aznabakieva conducted greenhouse visits and surveys, collected samples, and contributed to mycological analyses. Dr. Dilshod Ruzmetov carried out mycological analysis and pathogenicity tests. Prof. Anvar Sherimbetov and Dr. Bakhtiyor Adilov conducted molecular genetic experiments and contributed to finalizing the manuscript.

REFERENCES

Altinok, H. H., Yüksel, G. and Altinok, M. A. 2020. Pathogenicity and phylogenetic analysis of *Fusarium oxysporum* f. sp. *capsici* isolates from pepper in Turkey. Canadian J. Plant Pathology, 42: 279-291. DOI: 10.1080/07060661.2019.1641749.

Cerkauskas, R. F. 2017. Etiology and management of Fusarium crown and root rot (*Fusarium oxysporum*) on greenhouse pepper in Ontario, Canada. Canadian J. Plant Pathol., 39: 121-132. https://sci-hub.se/10.1080/07060661.2017.1321044.

Collins, C. 2020. Fungicides for control of damping-off in vegetables. https://draftlessig.org/which-fungicide-is-used-against-damping-off-of-seedlings-of-vegetables/.

El-kazzaz, M. K., Ghoneim, K. E., Agha, M. K. M., ***et al***. 2022. Suppression of pepper root rot and wilt diseases caused by *Rhizoctonia solani* and *Fusarium oxysporum*. Life, 12: 1-15. https://doi.org/10.3390/life12040587.

Engalycheva, I., Kozar, E., Frolova S., ***et al***. 2024. *Fusarium* species causing pepper wilt in Russia: Molecular identification and pathogenicity. Microorganisms, 12: 1-18. https://doi.org/10.3390/microorganisms12020343.

FAOSTAT, 2019. http://www.fao.org/faostat/en/#data/QC.L.

Gabrekiristos, E., Demiyo, T,. Teshome, D., and Ayana, G. 2020a. Hot Pepper Fusarium Wilt (*Fusarium oxysporum* f. sp. *capsici*): Epidemics, Characteristic Features and Management Options. J. of Agricultural Science, 12: 347-360. Published by Canadian Center Sci. Education. DOI:10.5539/jas.v12n10p347.

Gabrekiristos, E., Teshome, D., and Ayana, G. 2020b. Cultural, morphological and pathogenic variability among isolates of *Fusarium oxysporum* f. sp. *capsici* causing wilt of hot pepper in Central Rift Valley, Ethiopia. Plant Pathol. Microbiol., 11 (492): 1-12. DOI: 10.35248/2157-7471.20.11.499.

Gabrekiristos, E., Teshome, D., and Ayana, G. 2020c. Prospects of host resistance and biocontrol agent for the management of hot pepper Fusarium wilt (*Fusarium oxysporum* f. sp. *capsici*) in the Central Rift valley of Ethiopia. Acad. Res. J. Agri. Sci. Res., 8: 156-163. ISSN: 2360-7874. http://www.academicresearchJ.s.org/ARJASR/Index.htm.

Gholve, V. M., Tatikundalwar, V. R., Suryawanshi, A. P., and Dey, U. 2014. Effect of fungicides, plant extracts /

botanicals and bioagents against damping off in brinjal. African J. of Microbiology Research, 8: 2835-2848. DOI: 10.5897/AJMR2013.6336.

Gilardi, G., Matic, S., Gullino, M. L., and Garibaldi, A. 2019. First report of crown and root rot caused by *Fusarium oxysporum* on sweet pepper (*Capsicum annuum*) in Italy. Plant Disease, 103: 2946. https://doi.org/10.1094/PDIS-04-19-0863-PDN.

Grabowski, M. 2018. How to prevent seedling damping off. University of Minnesota Extension. Reviewed 2024. https://extension.umn.edu/solve-problem/how-prevent-seedling-damping.

Horst, R. K. 2013. Damping-off. Westcott's Plant Disease Handbook. Springer Netherlands, Dordrecht, p. 177. https://doi.org/10.1007/978-94-007-2141-8_25.

Khan, K. A., Nabi, S. U., Bhat, N. A., and Bhat, F. A. 2018. Chilli wilt disease: a serious problem in chilli cultivation in India. Indian Farmer, 5: 988-991. https://scholar.google.com/citations?view_op=view_citation&hl=en&user=IkUYls0AAAAJ&citation_for_view=IkUYls0AAAAJ:eQOLeE2rZwMC.

Khasanov, B., Khakimov, A., Khamiraev, U., ***et al***. 2021. Genus *Capsicum* L. and Diseases of Sweet and Hot Peppers (Review). Bulletin of Science and Practice, 7(10): 98-114 (in Russian, with English summary). https://doi.org/10.33619/2414-2948/71/1.

Lamichhane, J. R., Dürr, C., Schwanck, A. A., ***et al***. 2017. Integrated Management of Damping-off diseases. A review. Agron. Sustain. Dev., 37: 10. DOI 10.1007/s13593-017-0417-y.

Leslie, J. F., Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Ames, Iowa, USA, Blackwell Publishing, 388 pp.

Lomas-Cano, T., Boix-Ruiz, A., García-Rodríguez, C., ***et al***. 2016. Etiological and epidemiological concerns about pepper root and lower stem rot caused by *Fusarium oxysporum* f. sp. *radicis-capsici* f. sp. nova. Phytoparasitica, 44: 283-293. DOI: 10.1007/s12600-016-0522-5.

Lomas-Cano, T., Palmero-Llamas, D., Cara, M. de, ***et al***. 2014. First report of *Fusarium oxysporum* on sweet pepper seedlings in Almería, Spain. Plant Disease, 98: 1435. http://dx.doi.org/10.1094/PDIS-04-14-0365-PDN.

Lombard, L., Sandoval-Denis, M., Lamprecht, S. C., and Crous, P. W. 2019. Eutypification of *Fusarium oxysporum* – clearing the taxonomic chaos. Persoonia, 43: 1-47. https://doi.org/10.3767/persoonia.2019.43.01.

López-Orona, C. A., Hernández-Verdugo, S., Velarde-Félix, S., *et al*. 2019. Cross pathogenicity of *Fusarium oxysporum* isolated from peppers. European J. of Plant Pathology. Published online 02.04.2019. https://doi.org/10.1007/s10658-019-01732-z.

Meadows, I., Sharpe, S., and Henson, M. 2020. Damping-off in flower and vegetable seedlings. North Carolina State Extension. https://content.ces.ncsu.edu/damping-off-in-flower-and-vegetable-seedlings#.

Melanson, R. A. 2022. Damping-off in vegetables. Mississippi State University Extension. https://extension.msstate.edu/sites/default/files/publications/publications/P3747_web.pdf.

Mmbaga, M. T., Gurung, S., and Maheshwari, A. 2018. Screening of plant endophytes as biological control agents against root rot pathogens of pepper (*Capsicum annum* L.). J. of Plant Pathology and Microbiology, 9: 1-8. DOI: 10.4172/2157-7471.1000435.

Munene, G. 2022. How to achieve high sweet pepper yields. April 23, 2022. https://www.monitor.co.ug/uganda/magazines/farming/how-to-achieve-high-sweet-pepper-yields-3791992.

O'Donnell, K., Ward, T. J., Robert, V. A. R. G., *et al*. 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions. Phytoparasitica, 43: 583-595. DOI: 10.1007/s12600-015-0484-z.

O'Donnell, K, Whitaker, B. K, Laraba, I, *et al*. 2022. DNA sequence-based identification of *Fusarium*: A work in progress. Plant Disease, 106: 1597-1609. DOI: 10.1094/PDIS-09-21- 2035-S.

Oljira, T., and Berta, S. 2020. Isolation and characterization of wilt-causing pathogens of local growing pepper (*Capsicum annuum* L.) in Gurage zone, Ethiopia. Hindawi International J. Agronomy. Article ID 6638683. https://doi.org/10.1155/2020/6638683.

Pandey, A. K., Burlakoti, R. R., Rathore, A., and Nair R. M. 2020. Morphological and molecular characterization of *Macrophomina phaseolina* isolated from three legume crops and evaluation of

mungbean genotypes for resistance to dry root rot. Crop Protection, 127, #104962. https://doi.org/10.1016/j.cropro.2019.

Pandita, D., Bhat, M A., Mir, S., *et al*. 2019. Screening of traditional chilli cultivars of Kashmir for Fusarium wilt resistance. International J. Chemical Studies, 7: 1501-1503. https://www.chemiJ..com/archives/2019/vol7issue1/PartZ/7-1-113-485.pdf.

Pérez-Hernández, A., Serrano-Alonso, Y., Aguilar-Pérez, M. I., *et al*. 2014. Damping-off and root rot of pepper caused by *Fusarium oxysporum* in Almería province, Spain. Plant Disease, 98: 1159. https://doi.org/10.1094/PDIS-02-14-0212-PDN.

Romakaeva, K. 2021. Uzbekistan notes increase in bell pepper export. March 12, 2021. https://en.trend.az/casia/uzbekistan/3394281.html.

Sanogo, S. 2003. Chile pepper and the threat of wilt diseases. Online. Plant Health Progress. DOI:10.1094/PHP-2003-0430-01-RV.

Shaheen, N., Khan, U. M., Azhar M. T., *et al*. 2021. Genetics and Genomics of Fusarium Wilt of Chilies: A Review. Agronomy, 11: 2162. https://doi.org/10.3390/agronomy11112162.

Tamura K., Stecher G., Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38 (7): 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tsitsigiannis, D. I., Antoniou, P. P., Tjamos, S. E., and Paplomatas, E. J. 2008. Major diseases of tomato, pepper and eggplant in greenhouses. The European J. of Plant Science and Biotechnology, 2, Special issue No. 1: 106-124. http://www.globalsciencebooks.info/Online/GSBOnline/images/0812/EJPSB_2(SI1)/EJPSB_2(SI1)106-124o.pdf.

Velarde-Félix, S., Garzón-Tiznado, J.,A., Hernández-Verdugo, S., *et al*. 2018. Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Canadian J. of Plant Pathology, 40: 238-247. https://doi.org/10.1080/07060661.2017.1420693.

Velásquez-Valle, R., Amador-Ramírez, M. D., Medina-Aguilar, M. M. y Lara-Victoriano, F. 2007. Presencia de patógenos en almácigos y semilla de chile (*Capsicum annuum* L.) en Aguascalientes y Zacatecas, México. Revista Mexicana de Fitopatología, 25: 75-79. https://scielo.org.mx/scielo.php?script=sci_arttext&pid=S0185-33092007000100010.