ISOLATION OF PHYTOPATHOGENIC AND ENDOPHYTIC FUNGI FROM THE NEWLY CREATED FOREST PLANTATIONS ON THE DRAINED BOTTOM OF THE ARAL SEA

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Abstract

Salinization and drought are the most important abiotic stress factors causing significant impact to the agriculture of Uzbekistan. To eliminate the negative consequences of the drying of the Aral Sea, large-scale works are currently being conducted on this territory to create protective forest plantations of halophytic trees and shrubs.

An important issue in the protection of forest plantations is the isolation identification, and monitoring of phytopathogenic and endophytic fungi associated with these forest plantations.

As a result of the study, 35 strains of endophytic fungi were isolated from plant samples of black saxaul - *Haloxylonaphyllum* (Minkw.) Iljin, and two tamarix species –*Tamarixhispida*Willd., *Tamarixramossisima* Ledeb. The dominant species were 7 species: *Alternariatenuissima* (Kunze) Wiltshire, *Trichodermaviride* Pers., *Ulocladiumconsortiale* (Thiim) E.G. Simmons, *Acremonium* sp., *Chaetomium* sp., *Stemphylium* sp., *Alternaria* sp.

Pure cultures of fungal strains isolated within the framework of this study were deposited in the "Collection of a unique object of phytopathogenic and other microorganisms of the Academy of Sciences of the Republic of Uzbekistan" at the Institute of Genetics and plant experimental biology of the Academy of Sciences of the Republic of Uzbekistan.

Isolates of endophytic and phytopathogenic fungi obtained in this study can be used in the future for an in-depth investigation of their biological properties, development of measures to control fungal diseases of forest plantations and identification of new biologically active substances for agriculture and biotechnological use.

Keywords: soil salinization, halophytic plants, phytopathogenic fungi, endophytic fungi

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INTRODUCTION

Salinity and drought are considered to be the most important environmental abiotic stresses [1]. It is estimated that more than 900 million hectares (over 6%) of agricultural land and 30 percent of irrigation water worldwide are exposed to the influence of salinity [2].

Desiccation of the Aral Sea is considered to be the dramatic ecosystem collapse in central Asia. Once the fourth largest lake in the world with an area of 68,000 km^2, the Aral Sea has been steadily shrinking since the 1960s, after decades-old water diversions for irrigation [3] Today, this lake has lost 90% of its area.

As a result of the drying up of the Aral Sea, a complex set of problems have arisen with far-reaching global consequences. On the dried-up part of the sea, vast saline areas appeared, which formed the new so-called "Aralkum" desert. Its area comprises 5 million hectares, of which about 2.5-3 million hectares belongs to the Republic of Karakalpakstan.

In recent years much attention has been paid to halophytes which naturally adapted to survive and capable of completing their life cycle in saline habitats [4,5].Planting protective plantations using local halophytic trees and shrubs that can grow in highly saline soils and survive in arid conditions is an effective method of combating salt-dust transfer, as well as for fixation of drifting sands. Therefore, currently, large-scale works are being conducted on the drained bottom of the Aral Sea to create protective forest plantations and specialized forest nurseries.

In order to improve the efficiency of afforestation, it is necessary to conduct mycological monitoring to determine the composition and biology of phytopathogenic and endophytic fungi. Among the numerous diseases in the forestry, the first place in terms of prevalence and inflicted damage belongs to fungal infections. According to the frequency of their damage to plants, sporadic, focal and epiphytotic diseases are distinguished [6]. Massive outbreaks of disease in tree nurseries, which occur from year to year in certain areas, are caused by fungi that live and persist in the soil and plant debris and infect healthy plants. Pathogens with airborne spread and an iterative reproductive cycle during the growing season of plants are also known. Phytopathogenic fungi affect the leaves, trunks and root system of seedlings and adult plants.

On the other hand, there are also beneficial interactions between plants and fungi. Endophytic fungi are considered to be the functionally essential members of the plant microbiome [7] causing no detectable damage or disease symptoms in their host [8]. Although some endophytes could be evolved from phytopathogenic fungi (and vise versa) and may cause disease of plants under stress conditions [9], the true endophyte state is regarded as non-pathogenic long-term beneficial association.

The association of endophytic fungi with plants improves plant growth, their resistance to environmental stresses such as drought, salinity, temperature, heavy metals, etc., as well as resistance to pathogens [11, 12, and 13]. Endophytes isolated from plants growing in hot climates and saline soils have a high potential for practical application in the field of increasing stress resistance and productivity in conditions of high temperature and soil salinity [10].

The aim of this study is to isolate and identify phytopathogenic and endophytic fungi associated with halophytic plants growing on the dried bottom of the Aral Sea.

MATERIALS AND METHODS

For the analysis, we used plant samples of black saxaul –*Haloxylonaphyllum* (Minkw.) Iljin, and two tamarix species –*Tamarixhispida* Willd., *Tamarixramossisima* Ledeb. Which were collected during the expedition to the dry seabed of the Aral Sea.

Before sterilization, plant samples were thoroughly washed with running tap water to remove particles of sand, soil, and other debris. The roots were subjected to a three-stage surface sterilization procedure, treated with Tween 80 solution (200 µl in 100 ml of distilled water) for 10 minutes, 70% ethanol for 15 minutes, and twice with 2% sodium hypochlorite solution for 15 minutes, followed by rinsing with distilled water.

After these pre-treatment stages, roots were especially cuted into fragments with a length of 1.5-2 cm. Disinfected cuted plant fragments were transferred to Petri dishes 8 cm in diameter containing agar media (PDA, PCA, WA) and incubated at 25 ± 1 ° C in an artificial climate chamber for 4 weeks.

All fungi that grew from inside root samples were then transferred to media in order to isolate pure monocultures.

For long-term storage of the collection of field fungal isolates obtained in this study, the freezing method in glycerol (20%) at -20 ° C was used [14,30]. Conidia and conidiophores of each isolate were collected from agar cultures using a sterile needle and placed in sterile 1.5 ml Eppendorf

tubes containing 1 ml of 20% glycerol. These tubes were then stored at -20 ° C in the freezer.
Transient preparations were prepared by using methylene blue, crystal violet and iodine-glycerol from the isolates and photographed under a binocular microscope Nover (NLCD-307B). The shapes and sizes of macroconidia and microconidia were measured and x40, x100, x400 images of cells and mycelium were photographed.
Morphological identification of isolated fungi was conducted based on characteristics of the macro-conidia, phialides, microconidia, chlamydospores and the colony color and growth rate [16,18-20]

RESULTS AND DISCUSSION

As a result of the current study, 35 strains of fungi were isolated. The species composition of fungiwaswere very diverse. The dominant species were 7 species (belonging to 3 families and 5 genera): *Alternaria tenuissima* (Kunze) Wiltshire, *Trichoderma viride* Pers., *Ulocladium consortiale* (Thiim) E.G. Simmons, *Acremonium* sp., *Chaetomium* sp., *Stemphylium* sp., *Alternaria* sp. Below is a description of the cultural and morphological features of the first 2 species of isolated fungi:

Alternaria tenuissima (Kunze) Wiltshire - on potato agar forms a brown-black aerial mycelium, substrate mycelium - black. The surface of the colony is even, the edges are uneven, fast-growing. Under the microscope: Mycelium hyphae have transverse septa, which is characteristic of higher fungi of the Dothideomycetes class. Conidia are formed from 4-6 septa in transverse segments and have an apical secondary conidiophores. CLA is a poorer medium in composition, in which we observed the appearance of moderately abundant sporulation of fungi in comparison with the PDA medium, in which the formation of densely overgrown mycelium was observed. Also, microscopy revealed partial formation of apical outgrowths.
Trichoderma viride Pers. - on PDA forms a gray-green aerial mycelium, the surface of the colonies is uneven, with an uneven edge, the colonies are oval-shaped, fast-growing, the substrate mycelium is gray-green. Under the microscope: Conidia are unicellular, spherical in shape. At the ends of branched conidiophores, conidia are collected in heads of 10-20 pieces. Chlamydospores are spherical.

Figure 1 *Tamarix hispida* plants, healthy on the left, diseased on the right

Figure 2*Haloxylon aphyllum*plants, healthy on the left, diseased on the right

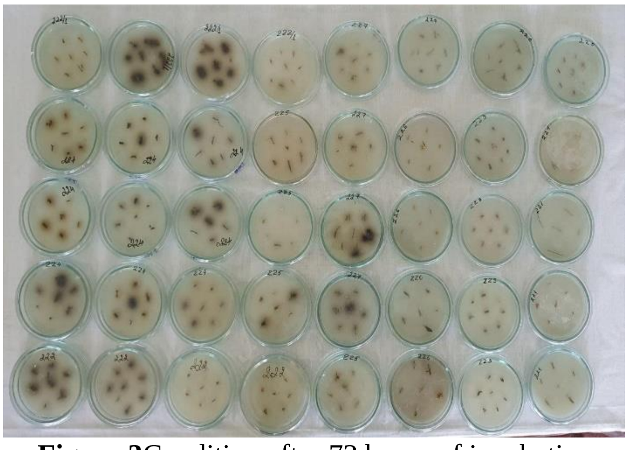

Figure 3 Condition after 72 hours of incubation

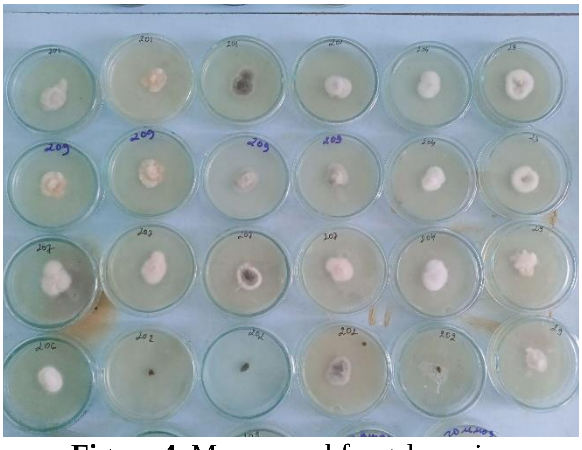

Figure 4. Monosporal fungal specimens

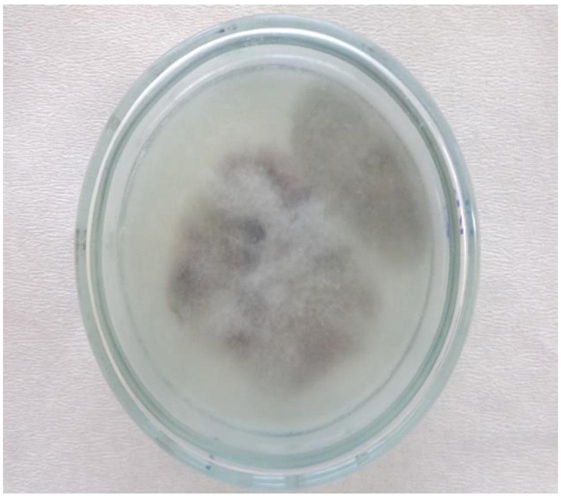

Figure 5. Alternaria tenuissima on the PCA media

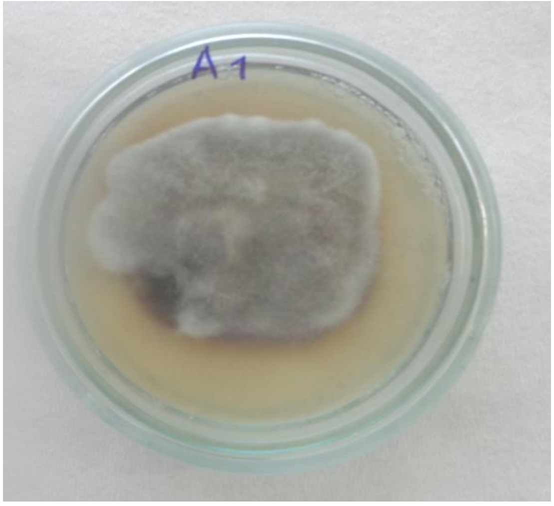

Figure 6. Alternaria tenuissima on the PDA media

Рис. 7. Conidia of Alternaria tenuissima on PCA medium (magnification x400)

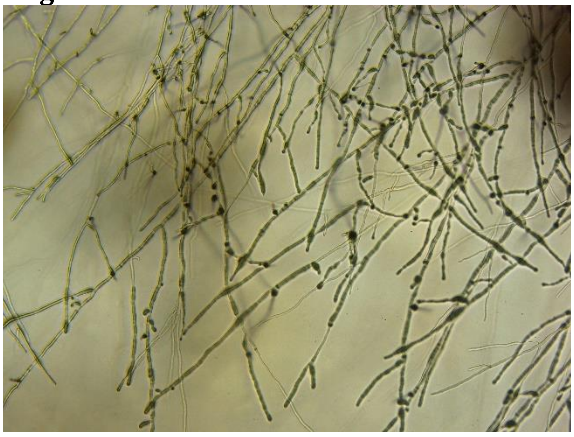

Рис. 8. Mycelium of Alternaria tenuissima on PCA medium (x100 magnification)

Table 1. The occurrence of endophytes in the investigated plants

№	**Plantname**	**Totalinvestigatedplant segments**	**Number of fungal isolates**	**Occurrence in %**
1	*Haloxylonaphyllum*	42	61	32
2	*Tamarixhispida*	23	34	12
3	*Tamarixramossisima*	28	47	17

The analysis of the distribution of fungi in plant organs showed that they populate differently on different plant organs, for example, as shown in the diagram, 24.7% were isolated from the roots, 36.6% from stems, 16.2% from leaves, 3.5%fromflowers, 19% from fruits.

Fungi of the genus *Alternaria* are plant pathogens that capable of infecting plants at all stages of development - from seeds to adult plants and fruits. According to the current data, about 280 species of fungi of the genus *Alternaria* have been characterized in the world, from which 50 species cause economically significant diseases [16]. Growing trend of the economic damage to the agriculture caused by fungi of the genus Alternaria is observed in all regions of the world.

Results of our study are consistent with a previous work performed by Yarmolovich V. A. et al. in which it has been showed, that the most common diseases in forest nurseries of Belarus are caused by *Cladosporium and Alternaria spp.*[21].

Also our results are consistent with in a recent study performed by Kasun M. Thambugala et al. [22] in which *Alternariatenuissima* reported for the first time on Tamarix spp.

It should be noted that recently, severe disease outbreaks in the population of *Ailanthus excelsa* caused by *Alternariaalternata* were recorded in the nursery of the Forest Research Institute, Dehradun. *Ailanthus excelsa*is very drought resistant tree and native to India [23].Previous research conducted in the Iraq performed by Tawfik M. Muhsin revealed twelve species of Alternaria that were isolated from six salt marsh halophytes, *Atriplexpatula, Glauxmaritima, Hordeumjubatum, Puccinellianuttalliana, Salicorniaeuropaea, Suaedadepressa,* and it has been suggested that the ability of A. alternata to tolerate a wide range of salinity may allow this fungus to survive under stress conditions and it has been envisaged that probably the association of *Alternaria* species with stress-tolerant plants may be advantageous from the ecological perspective[24].It is an interesting phenomenon and its molecular mechanisms remain to be investigated. Some fraction of *Alternaria* species are considered to be a common saprophytes that could coexist peacefully with their host and only start to cause disease when the right conditions occur or when they have acquired pathogenic capacities by mutations or horizontal gene transfer.

It should also be noted that some endophytic fungi associated with plants in natural ecosystems help plants overcome abiotic stress factors such as soil salinity, drought, and high temperature [15]. Plants growing on the drained bottom of the Aral Sea are exposed to severe salt stress. The ability to withstand the stress of high salinity is essential for survival in this environment. Thus, it is reasonable to suggest that some strains of fungi isolated in this study, such as *Trichodermaviride Pers*. contribute to the survival and growth of their host plants by increasing their resistance to drought and salt stress. It should be noted that fungi of the genus *Trichoderma* are capable of producing several phytohormones and effector-like molecules [25,26, 27, 28, 29]. Phytohormones are required for many processes of plant development, including seed germination, stem elongation, leaf expansion, induction of flowering, maturation, and the development of resistance to abiotic factors [17].

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Isolates of endophytic and phytopathogenic fungi obtained in this study can be used in the future for a deeper study of their biological properties, development of approaches to control fungal diseases of forest plantations in the salt-affected arid regions and identification of new biologically active substances for agriculture and biotechnological use.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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