Propagation of fungi in the soils: A case study of scientific experimental fields of forestry of Tashkent region

\textbf{S Yu Khidirov\textsuperscript{1}, R Gulmurodov\textsuperscript{2*}, M S Mamiev\textsuperscript{3}, and D Yuldosheva\textsuperscript{1}}

\textsuperscript{1}Research institute of Forestry, 111104 Tashkent, Uzbekistan
\textsuperscript{2}Department of Agricultural Phytopathology, Tashkent State Agrarian University, University str. 2, 100140 Tashkent, Uzbekistan
\textsuperscript{3}Department of Agrobiotechnology, Tashkent State Agrarian University, University str. 2, 100140 Tashkent, Uzbekistan

\textsuperscript{*}E-mail: gulmurodov70@mail.ru

\textbf{Abstract.} The sphere of forestry in our republic is developing in the current years. For the purpose of landscaping the territory of our country, reproduction and cultivation of virginia firs, abina firs, pine species, cypress trees, eastern biota, western camel, spruce and other deciduous trees from coniferous trees is widely established. These ornamental trees differ from other ornamental trees and shrubs in their heat–cold, drought resistance. Different landscapes and forest trees are initially planted in germination plants and seedlings from their seeds. In Uzbekistan, diseases of decorative and forest trees, especially their diseases in the germination period, have almost not been studied. The fact that many different diseases are encountered in landscape trees in recent years is known from different sources. The article provides information on the influence of the seasons on the spread of fungi that have appeared on the soil of some plantations and seedlings in Tashkent region.

1. Introduction

The total land area of the Republic of Uzbekistan is 44896.9 thousand hectares, of which 25 percent of the total land area of the republic, that is, more than 11242.3 thousand hectares, corresponds to the forest fund. The main part of the forest fund is 81 percent in the desert zone, 16 percent in the mountainous areas, 3 percent in the valleys and rivers. The big problems facing the forestry sector of our country are waiting for their solution, they are increasing the level of forest cover, expanding the areas of juniper forests, enriching the dendroflora of our country with new species, reducing the harmful effects of the drained Aral Sea on the environment and others.

Most of the biochemical changes that occur in nature and in the soil occur with the participation of microorganisms. Let’s not take into account what process takes place in the soil, we are sure that they are closely related to the activity of microorganisms. Around the root of plants is rich in a variety of microorganisms, these microorganisms have a great influence on the development and nutrition of plants, mastering the substances allocated by the root of the plant and changing various organic and mineral substances around the root \cite{1}. Forests of Central Asia, forest growth conditions are radically different from the forests of Russia, Ukraine, Belarus in terms of dendrological composition and economic activity. Forests of our country perform mainly protective functions \cite{2}.
In the cultivation of decorative and forest trees, quality seeds of various fungi are of great importance. Many fungi in the seeds are saprophytes, they do not affect the seed quality, but can cause damage to the soil with pathogenic fungi. The most studied fungi are in coniferous, they are species of the category fuzarium, for example F.circinatum, F.oxysporum and Pensillium, Aspergillus, Pestalotia, Trichoderma, Mucor, Rizopus and others [3].

The species of the category Fuzarium forms a colony very quickly in the soil and can remain motionless, like chlamydia spores, and may not develop until the host plant appears [4]. Fungi of the Trichoderma category are typical representatives of different soils. This category of fungi is used in agriculture against phytopathogen fungi [5,6].

In increasing soil fertility and metabolism in it, fungi plays an important role in the accumulation of mineral nutrients necessary for forest plants, in the synthesis of organic substances as well as a number of microorganisms. It was observed that the occurrence of fungi and their amount depends on the climatic conditions of the soil, plant species, pH indicator of the soil, soil aeration, humidity, temperature, depth of the layer, the season of the year, the period of growth of plants, the degree of soil assimilation and many other factors [7,8].

2. Materials and Methods

In our research, Tashkent Regional Forestry Department of the State Committee of Forestry “Ohangaron” state forest department (dark brown-gray soil), state forest production enterprise “Saksonota” (dark brown-gray soil), specialized State Department of Forestry Seedlings “Kuyi Chirchik” (hydromorph meadow gray soil), State Forest Department “Shirinsay” of Bekabad district (typical gray soil) and scientific experimental department “Darkhon” under the Forestry scientific-research institute (typical gray soil) and we used soil samples from seedlings. Soil samples samples were taken from 0-10, 10-20, 20-30 cm depth layers (under sterile conditions) in all seasons of the year, and they were analyzed mycologically in the laboratory.

More than 70 species are grown in “Darkhon” scientific experimental farm, more than 45 species in “Shirinsoy” forest department, more than 60 species in “Ohangaron” state forestry enterprise and more than 50 species of decorative and seedlings of forest trees are grown in “Saksonota” state forest production enterprise.

Soil samples were taken from 3 places of seedlings: from the center, as well as from two outskirts (in 3 repeats) in sterilized packages.

In order to obtain the samples, the soil samples were first taken with a sterilized knife, which was previously prepared in the range of every 10 cm, digging depth of 30 cm. Soil extraction began from the bottom layer.

In carrying out the research, methods adopted in microbiology and mycology were used, that is, methods of soil dilution, formation of a moisture chamber, planting and separation of fungi.

Method of sowing small particles of soil: for the separation of fungi, small particles of soil were evenly sprinkled on the surface of the nutrient medium Petri saucers and placed in a thermostat with a temperature of 24-26°C. After 3-7 days around the soil fragments, colonies of various fungi appeared. The sprouted fungi were planted in the agaric nutrient medium of the tube by means of a mycological hanger.

Soil liquefaction method: soil liquefaction was carried out on the basis of the method adopted in microbiology and mycology the day before the sample was taken [9]. In order to calculate the total amount of fungi, 10 g of soil was poured into 90 ml of sterilized water and mixed in a magnetic mixer (IKA C-MAG HS7) for 10-15 minutes. With the help of a sterilized pipette, 1 ml of suspension was placed in 9 ml of sterilized water in the tube. This process was repeated several times. The suspension in the third and fourth tubes (1:1000, 1:10000) was sown from 0.33 ml using a sterile pipette to the pre-prepared nutrient medium Petri saucers, that is, 1 ml of the suspension was sown as three Petri saucers in the nutrient medium. Soil suspension, planted in Petri saucers, was spread evenly over the surface of the nutrient medium using a sterile spatula. Then the petri dish was put in a thermostat with a temperature of 24-26°C.
In order to determine the amount of fungi in 1 g of absolute dry soil, from the soil sample taken simultaneously weighed on 1 g of soil weight and dried. The amount of fungi in 1 g of soil was determined by the following formula:

\[ a = \frac{b \times c \times e}{d}, \]

where:
- \(a\) - cell content in 1 g dry soil, on the account of pieces
- \(b\) - the average amount of colonies in the saucer, on the account of pieces
- \(c\) - the amount of liquid planted, on the account of ml
- \(e\) - The amount of 1 ml suspension, on the account of drop
- \(d\) - dry soil weight taken for inspection, g [10].

Method of forming a moisture chamber: the moisture chamber method was also used in separating soil fungi [11]. To do this, pieces of soil were placed in a sterile Petri saucer with a damp filter paper and placed in a thermostat with a temperature of 24-26°C. Fungi that sprouted from the soil were obtained in pure form, and the total amount and systematics were determined.

Method of planting and separating fungi: in order to encroach the total amount of fungi, \(\psi\)-agar, Chapek, potato agar, starchy food, as well as nutrient media used for the purpose of separating the Verticillium Nees et Lk were used [12]. Prevention of bacterial growth was obtained by adding citric acid or streptocide to the agaric nutrient medium as a pH indicator of the nutrient medium. Some fungi have been equated to PH 6.5-7.0 agaric nutrient medium without parallel, taking into account the development of a neutral and weak alkaline environment. Petri saucer, in which soil samples were planted, was stored for up to 15 days in a thermostat with a temperature of 26-28°C. Petri saucer samples were examined from the 3rd day of planting, fast-growing fungi were planted in tubes with agaric nutrient medium. Observation lasted up to 15 days.

Preparation of the nutrient medium: extraction of pure cultures of fungi from samples obtained from the soils of forestry, where observation works were carried out, from artificial nutrient media prepared on the basis of the methods used in mycology. Chapek nutrition, potato agar nutrition was sown in beer wort and in a moisture chamber. The composition of this nutrient medium is as follows: wort agar nutrient medium - 1L of non-alcoholic beer wort, 20 g of agar-agar, 2 l of water. Chapek nutrient medium-20 g of sucrose, 2 g of \(\text{NaNO}_3\), 1 g of \(\text{KH}_2\text{PO}_4\), 0.5 g of \(\text{MgSO}_4\), 0.5 g \(\text{KCl}\), 20 g of agar-agar, 1 l of water. Potato-agar nutrient medium-prepared nutrient medium consisting of 200 g of purified potatoes, 20 g of sucrose and 20 g of agar-agar [13,14].

### 3. Results and Discussions

As a result of scientific research conducted by scientists working in the same field, the amount of fungi and other micro-organisms in the soil and the laws of their species spread are clearly revealed. The spread of microorganisms in the soil is indicated by the season of the year, the depth of the soil horizon and the direct correlation with climatic conditions. Many scientists believe that in the soil there is an increase in the number and quantity of fungi in the spring and autumn seasons [15,16,17]. The main part of the landscape and forest trees germination and seedlings they die by drying under the influence of microorganisms on different soils in the germination and seedlings under cultivation. Useful microorganisms in the soil have a positive effect on the growth and development of plants [18], while phytopathogen microorganisms cause various diseases in them our experiments were carried out on the soils of the fields where Crimean Pine, Tulip Tree, Canadian crimson, Silk Acacia, Virginia juniper, Eldor Pine, Typical oak, Indian rosehip, large-leaved linden, false chestnut and other sprouts were planted. Here, quantity and species composition of mycoflora soils grown in landscape and forest tree germination and seedlings were studied in scientific experimental seedlings of forestry of Tashkent region.

In the conducted research, there were separated fungi belonging to 2 classes, 2 orders, 4 families, 9 categories from the germination and planting soils (Table 1).
Table 1. Systematic list of fungi isolated from soil

| Class           | Order      | Family     | Category                      |
|-----------------|------------|------------|-------------------------------|
| Zygomycetes     | Mucorales  | Mucoraceae | Mucor Mich. et Fr.            |
| Deuteromycetes  | Hyphomecetales | Moniliaceae | Aspergillus Mich ex Fr.       |
|                 |            |            | Penicillium Lk ex Fr.         |
|                 |            |            | Trichoderma Pers. Ex Fr.     |
| Dematiccaceae   |            |            | Alternaria Nees ex Lk        |
|                 |            |            | Cladosporium Lk ex Fr.       |
| Tuberculariace   |            |            | Fusarium Lk ex Fr.           |

In our conducted studies, it was observed that the amount of microorganisms in the soil varies depending on the seasons of the year. Although, when the maximum amount of fungi in the soil was observed in the spring (in 1 g of soil 103,6 thousand), its minimum amount was observed in winter (in 1 g of soil 52,4 thousand). In the summer and autumn seasons, their amount in 1 g of soil was 66,8 and 84,6 thousand (Table 2).

The most abundant fungi in all seasons of the year became Aspergillus and Penicillium fungi.

Table 2. The amount of fungi in the soil of seedlings and saplings house in the scientific experimental areas of forestry of Tashkent region (2018-2019)

| Fungus categories | Spring | Summer | Autumn | Winter |
|-------------------|--------|--------|--------|--------|
| Alternaria        | 1887   | 1499   | 2052   | 1252   |
| Aspergillus       | 65674  | 46357  | 50456  | 35149  |
| Cladosporium      | 3547   | 1262   | 3606   | 2705   |
| Fusarium          | 3964   | 3363   | 5127   | 959    |
| Penicillium       | 21897  | 9637   | 15676  | 8698   |
| Stachobotrys      | 1174   | 1249   | 2532   | 1428   |
| Stembhylium       | 1537   | 1614   | 2016   | 752    |
| Tchrichoderma     | 2998   | 1123   | 1980   | 931    |
| Mucor             | 953    | 758    | 1245   | 532    |
| Total:            | 103631 | 66862  | 84690  | 52406  |

In the spring, the fungus, belonging to the Aspergillus and Penicillium categories encountered more often than in other seasons of the year. Representatives of the Tchrichoderma series also met more often in spring than in other seasons of the year. In summer, fungi from the Aspergillus, Penicillium and Fusarium categories were more distinguished. The minimum amount of fungi belonging to Mucor category compared to other category of fungi was observed in summer.

In spring Aspergillus niger, A.versicolor, A.flavus, A.insultus, A.ochraceus, A.terreus, A.Ostos, Penicillium parpurogenum, P.fellutanum, P.rubrum, P.soppi, P.lilacinum, P.notatum, P.solfereum, Alternaria tenois, A.geophyslia, Fusarium moniliform, F.oxysporium met the maximum amount of fungi.

In summer, fungi from the Aspergillus, Penicillium and Fusarium categories were distinguished (see Figure 1). The minimum number of fungi in the category of Cladororium was recorded in summer.

In autumn, representatives of the Aspergillus category also took precedence in this season, when the amount of Alternaria, Fusarium, Cladosporium categories was most often encountered.
Many fungi which are met in autumn: *Aspergillus niger*, *A.flavus*, *A.terreus*, *A.Ostos*, *A.insultus*, *A.ochraceus*, *Penicillium lilacinum*, *P.purpurogenum*, *P.ruhrum*, *P.notatum*, *Fusarium javanicum*, *F.lateritium*, *F.moniliforme*, *F.axysporium*, *F.solani*, *Alternaria brassicae*, *A.geophilla*, *A.tenois*, *Cladosparium epiphyllum*, *C.herbarium*, *Stachobotrys cylindrospora*, *Tchrichoderma lignorum* fungi species were separated from the soil in all seasons of the year.

In winter, the minimum number of soil fungi was observed and the minimum number of fungi from the *Alternaria*, *Aspergillus*, *Penicillium* and *Tchrichoderma* categories was recorded in comparison with other seasons. The reason for the large number and variety of fungi in spring and autumn seasons is that the temperature and humidity are favorable for the development of fungi in these seasons.

**4. Conclusions**

In spring, summer, autumn and winter months of 2018-2019 it was determined that there are a large number of fungi in the soil in spring and autumn compared to winter and summer seasons in the plantations and seedlings in the fields of scientific experiment of forestry of Tashkent region. The amount of fungi and their species are directly related to the seasons of the year, regardless of the type of soils studied in the plantations and seedlings in the scientific experimental fields of forestry of Tashkent region.
Tashkent region, was determined. In spring and autumn period of the year, it was determined that their quantity and number of species were significantly higher than in other seasons of the year.

Conflicts of Interest
The authors declare no conflicts of interest.

References
[1] Mamiev MC 1997 Microflora of some types of soils of the Surkhandarya region and the Republic of Karakalpakstan, Candidate of Science Dissertation, Tashkent.
[2] Kayimov AK, Berdiyev ET 2012 Dendrology, Science and Technology Publishing House, Tashkent.
[3] Fräedrich SW, Michella M 2012 Conifer and Hardwood Diseases 39 132-134.
[4] James RL 2012 Conifer and Hardwood Diseases 34 117-120.
[5] Alimova FK 2005 Trichoderma/Hypocrea (Fungi, Ascomycetes, Hypocreales): taxonomy and distribution, Publishing House of Kazan University, Kazan.
[6] Schuster A, Schmol M 2010 App Microb Biotechnol 87(3) 787-799.
[7] Ibadov K 1973 Comparative study of the composition and some physiological and biochemical characteristics of micromycetes of plain and mountain soils of Uzbekistan, Candidate of Science Dissertation, Tashkent.
[8] Zuparov MA 1984 Comparative study of the mycoflora of the mulberry rhizosphere, Candidate of Science Dissertation.
[9] Litvinov MA 1969 Methods for studying soil microscopic fungi, The Science, Moscow.
[10] Zvyaginstev DG 1980 Methods of soil microbiology and biochemistry, Moscow University Publishing House, Moscow.
[11] Bilay VI 1973 Experimental mycology methods, Naukova Dumka, Kiev.
[12] Gulomova M, Ramazanova CC 1975 Algae and Mushrooms of Central Asia 2 173-175.
[13] Dudka IA, Vasser CP, Ellanskaya IA et al 1982 Experimental mycology methods, Naukova Dumka, Kiev.
[14] Morozov AI, Timoseev AA 2002 Mushroom cultivation, Mycelium, Publishing House ACT, Moskow.
[15] Orazov XN 1976 Microflora of some soils of the Turkmen SSR and antagonistic relationships of its representatives, Ilym, Ashgabat.
[16] Kashieva AK 1990 12th Association of the Plenum of the Council and the Republican Committees for the Progression of UNESCO "Man and the Biosphere", Frunze.
[17] Sheraliev A, Fusarium L 1995 The role of environmental conditions in the distribution of the fungus in phytocenoses, Control of plant pests, diseases and weeds, Tashkent State Agrarian University Scientific Collection, Tashkent, pp 54-66.
[18] Dobrowolski JW, Bedla D, Czech T, Gambus F, Gorecka K, Kiszczak W, Kuzniar T, Mazur R, Nowak A, Slivka M, Tursunov O, Wagner A, Wieczorek J, Siwatek M 2017 Integrated Innovative Biotechnology for Optimization of Environmental Bioprocesses and a Green Economy Optimization and Applicability of Bioprocesses eds Purohit H, Kalia V, Vaidya A, Khardenavis A (Singapore: Springer) chapter 3 pp 27-71.