Soil-borne plant pathogenic fungi biodiversity of sunflower

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Abstract. Diseases caused by soil-borne phytopathogenic fungi are a serious problem for agricultural crops, including sunflower crops. The limited possibilities of measures to control them further increases the interest and focus on them. This article reveals data on the species composition, occurrence rate of soil-borne plant pathogenic fungi isolated from the soil of sunflower. Here, 11 species of true fungi and 1 species of oomycetes have been isolated. The highest incidence rate was recorded in the *Fusarium oxysporum* fungi species. During the study, pathogenic fungi specific to sunflower have also been isolated.

Keywords: *soil-borne fungi, diseases, sunflower, fungus, Fusarium, Sclerotinia, Verticillium, Rhizoctonia, Pythium.*

1. Introduction

The largest number of microorganisms is associated with the soil – bacteria, actinomycetes, fungi, algae, invertebrates and others. Their total number varies in different soils, that is, ranges from a few hundred thousand to several billion cells per 1 g of dry soil. The biomass of soil microorganisms is 1 to 8 tons or more per 1 ha of arable land. Microorganisms living in the soil are actively involved in the processes of mineralization of organic matter, the formation of soil humus, the movement of nutrients, and so on. Soil fertility depends in many ways on their activity. Among the microorganisms in the soil there are a number of pathogenic species that cause disease in plants, including root damage: rot, wilting, root hypertrophy, "black spot" and others. Fungi are often the causative agents of root diseases. Soil is the main source of water and the source of infection for them. The life cycle of many phytopathogenic fungi is partially related to soil. This is typical for fungal species that cause rust, *Sclerotinia*, head rot and other diseases [1-5].

The control of soil-borne fungal diseases depends on an understanding of the interactions of complex species. Soil-borne pathogenic fungi are “hidden” in the soil, diversifying, causing a decline in crop yields and damage to the agricultural economy. Thus, soil phytopathogens can adversely damage the yield of many economically important crops. The introduction of crop rotation is one of effective ways to stop the plant-pathogen cycle, prevent the accumulation of soil-borne phytopathogenic fungi [6-9]. In addition, various agrochemicals have been developed to accelerate plant growth and reduce the impact of pathogenic fungi. However, due to the negative impact of many pesticides on the environment and strict regulation of their use [10], there is a need to further reduce pesticide use and identify more sustainable plant protection strategies [11].

Similar ideas on this regard have been studied in the development of a intercropping system in agriculture (i.e., the cultivation of several types of crops in a field) [12]. Intercrops typically yield 20% more than single crops grown in proportion [13]. Excessive yields may probably be partly due to better light absorption and nutrient availability, but due to reduced diseases in intercropping when compared potentially to monocultures As for the latter, analyzes show a reduction in soil-borne diseases such as root rot and Fusarium wilt [14, 15]. For example, in 30 of the 36 studies on intercropping farming systems, soil-borne diseases were reduced [16, 17].

In recent years, the quantity and quality of agricultural crops have been declining under the influence of adverse microorganisms. This is due to the fact that pathogenic microorganisms adapt easily to climatic conditions and effective control measures are not carried out in a timely manner. Development and implementation of modern measures to combat pathogenic microorganisms will allow to obtain high and quality yields of agricultural crops [18, 19].

To date, the species and biology of soil-borne phytopathogenic fungi in sunflower growing areas have been little studied. This is because the main focus of many studies on sunflower phytopathogens has been on terrestrial pathogens [20-22].

Increased attention to terrestrial diseases in sunflower fields is due to the fact that they are often easier to observe and study. In addition, many terrestrial phytopathogens can cause serious epidemics due to their rapid and long-distance (up to kilometers) spread in the air [23, 24]. In contrast, the effects of underground soil-borne phytopathogenic fungi are mainly capable of spreading over short distances (from cm to maximum meters per year) [25].

According to Lukomets and Piven [26], sunflowers can be returned to the previous fields for cultivation after 8 years in crop rotation. This is primarily determined by the need to protect sunflowers from soil pathogens, broomrape, cottony rot and head rot, downy mildew, Fusarium wilt, as they can persist in the soil for a long time [27].

2. Materials and methods

In 2019-2021, we conducted our experiments in the fields of "Rayim aka" and "Surantkent Orzu Invest" farms in Kibray district of Tashkent region, on sunflower varieties "Dilbar", "Salyut" and "Jahongir" [28, 29]. In order to fulfill the scientific tasks set, we have widely used the methods developed by mycologists and phytopathologists and adopted for practice.

We used Waxman's soil dilution method to isolate phytopathogenic fungi from the soil. This method is used by most mycologists. This method is suitable for studying the fungal flora of different soils, the description of soils planted with different plants, the effect of mineral fertilizers or other agrotechnical measures on the species composition of fungi [30].

Analysis of soil microflora by soil dilution method was performed as follows. Soil samples taken from the sunflower planting area were placed in sterile test tubes or paper bags made of double-layered parchment paper. The obtained soil samples were passed through a fine sieve with a diameter of 4 mm and weighed by 10 g 3-4 times on a technical scale, then placed on a filter paper with a spatula. For each sample, a new filter paper was used and the spatula was washed with water, immersed in an alcohol container, and passed through a flammable alcohol flame. The soil samples were placed in a sterile flask containing 90 ml of sterile water and shaken for 5 min. Then the mixture was taken by 1 ml from the flask and poured into a sterile tube with 9 ml of water inside and shaken slightly. Similarly, 1 ml of the mixture from this tube was transferred to the next tube, and this process was repeated four times. From the fourth test tube, 1 ml of the mixture was inoculated into sterile Petri dishes. The mixture from each test tube were transplanted into a Petri dish in 4 replications. It was then filled with 10 ml of nutrient medium cooled to 40 °C and placed in a thermostat. They were grown in a thermostat at a temperature of 23–25 °C for 10–15 days (Figure 1). From the third day, they were examined and the colonies of fungi were counted, they were transferred to test tubes containing the nutrient medium. The final calculation was made after 15 days [31-34].

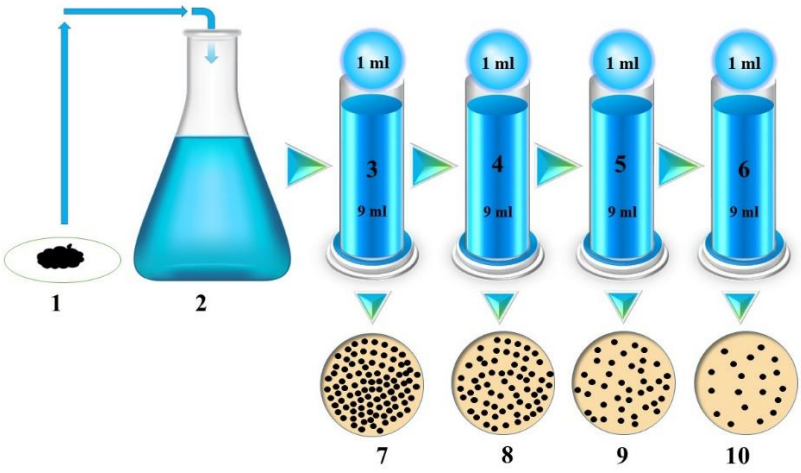

Figure 1. Growing process in a thermostat: *1- filter paper with 1- 10 g of soil. 2- flask with 90 ml of water in it. 3-6 – tubes filled with 9 ml of water. 7-10 – Petri dishes inoculated in different mixtures.*

Species composition of isolated fungi was determined with guides for fungi identification by Pidoplichko N.M. and Naumov N.A. et al. [35-37].

3. Results and discussion

During our research conducted in 2019-2021, we monitored the storage of phytopathogenic fungi in the soils of sunflower planted areas.

When we analyzed the three-year data, we found that most of the phytopathogenic fungi that cause disease in sunflowers are stored in the soil. The results of the study proved that the main source of infection of the diseases root rot, cottony rot and head rot, Fusarium and Alternaria leaf spot is soil.

The most common species of phytopathogenic fungi in sunflower were identified in the fields where sunflower was planted continuously. This means that phytopathogenic fungi are stored in the soil or plant residues and it is required proper crop rotation in this case. The largest number of identified species (33.3%) are fungal species belonging to the genus Fusarium. Species of Fusarium oxysporum was found in the roots, leaves, stems and head of sunflowers during the growing season (Table 1). Other representatives of the Fusarium genus were seen to cause more root rot of young seedlings and wilting of seedlings (Figure 2).

Table 1. Biodiversity of soil-borne phytopathogenic fungi.

№	Identified fungi species	Plant parts under infestation			
		root	leaf	stem	head
1	*Fusariumoxysporum*	+	+	+	+
2	*F. culmorum*	+			
3	*F. graminearum*	+			
4	*F. avenaceum*		+	+	
5	*F. solani*	+			
5	*Fusarium spp.*	+			+
6	*Sclerotinia sclerotiorum*	+		+	+
7	*Verticillium dahliae*	+			
8	*Rhizoctonia solani*	+			
9	*Pythium spp.*	+			
10	*Alternariahelianthi*	+	+	+	+
11	*Alternariaalternata*		+		+

12	*Thielaviopsisbasicola*	+			

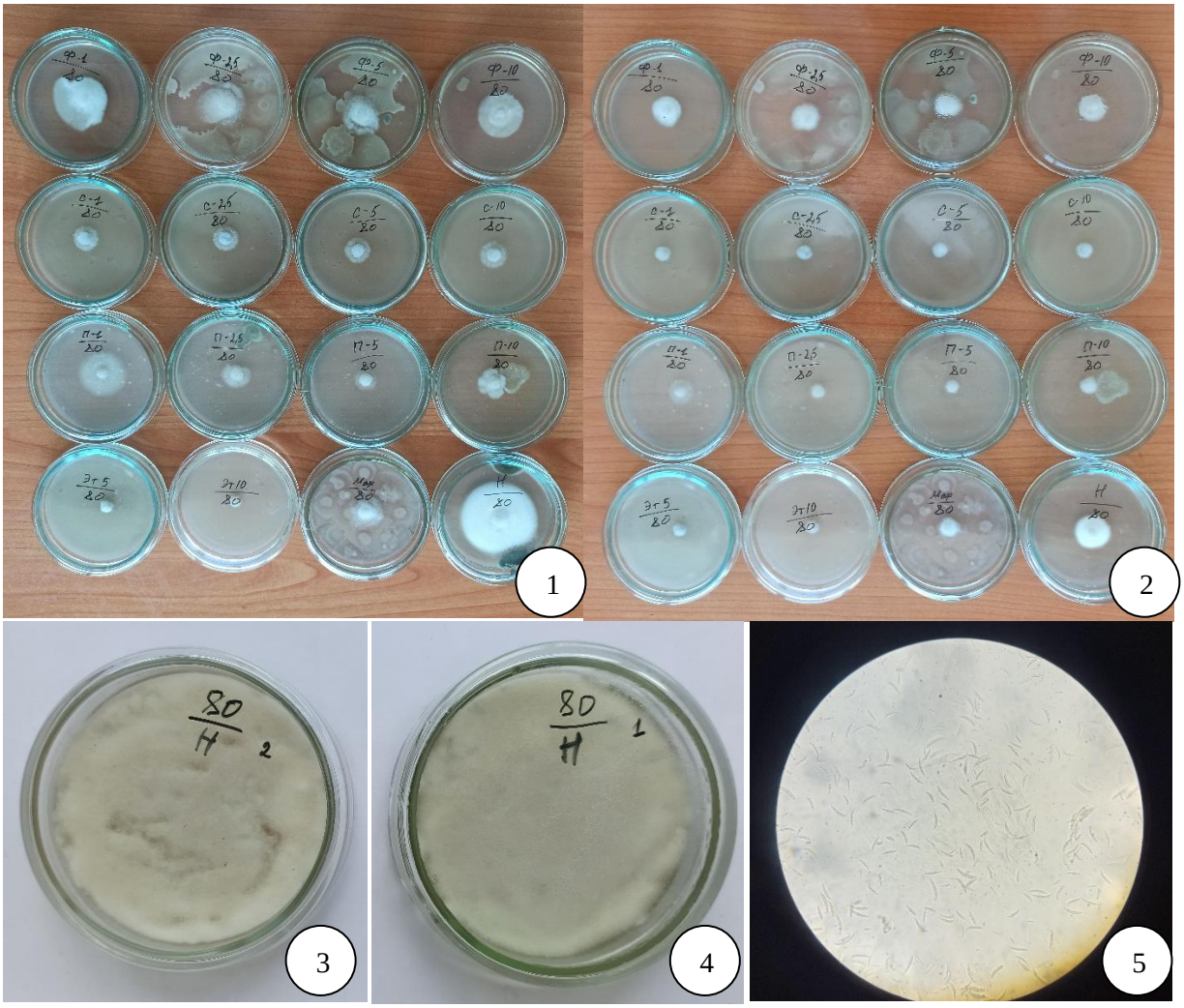

Figure 2. Fusarium genes under microscope: *1-2- Isolation of Fusarium oxysporum fungus by the method of soil dilution; 3-4- formation of fungus colonies; 5- microscopic view of macro and micro conidia*

According to A.A. Vypritskaya et al. [38], *Fusarium spp*. appears in sunflowers at all stages of plant development and causes damage to roots and all soil surface organs (rot, wilting, pink rot of the head, and death of seedlings) [39]. The high toxicity of *Fusarium* pathogens is maintained even when they are replanted into medium. [13, 18]. The severity of pathogens depends on their type, climatic conditions, time of damage and the number of infected plants [38].

The fungus *Sclerotinia sclerotiorum*, which causes cottony rot, was observed to remain in the soil as sclerotium [5, 30]. We observed that this fungus infects the root collar, stem and the head of sunflower. The fungus *Sclerotinia sclerotiorum* forms multicellular colorless mycelium in the infected organs of the plant. The sclerotia are round or slightly elongated and often form in the cavity of the sunflower stem. They are white at the beginning of their appearance, and when they are mature, they turn darker. Their size varies from 1-15 mm to 10-12 cm. The sclerotia located in the soil retain their viability for 3-5 years, while those on the soil surface survive for 1-2 years (Figure 3).

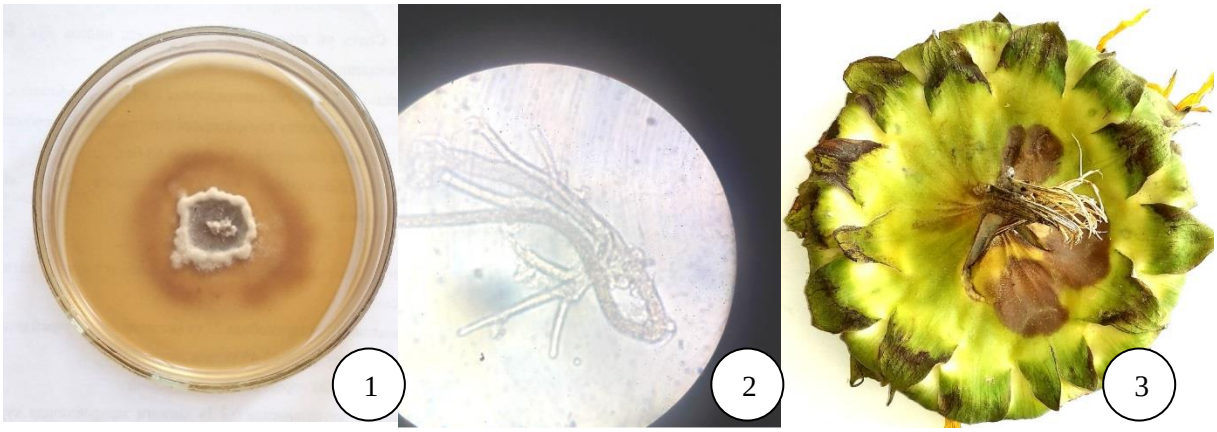

Figure 3. Sclerotia under microscope: *1- mycelium formed in the nutrient medium; 2-its microscopic view; 3-disease pathogen on sunflower head.*

The fungus penetrates into the tissues of sunflower and produces the enzymes pectinosterase and polygalacterase, as well as oxalic acid. Under their influence, hydrolysis of pectin and destruction of plant tissue are occurred [22].

The fungus *Sclerotinia sclerotiorum* forms mycelium, sclerotia, secondary sclerotia, microconidia and ascosporeal apothecium in the life cycle of its development. Plant infestation usually occurs through ascospores that rise into the air from the apothecium, or also from diseased plants nearby or through mycelium that spreads from sclerotia [21].

The phytopathogens of *Rhizoctonia solani, Verticillium dahliae, Thielaviopsis basicola (Berk. et Br.) Ferr.* and *Pythium spp.*fungi that cause root rot and root collar rot diseases in sunflower seedlings have been identified in all three years of the experiment. From this result it can be understood that these phytopathogens are pathogenic in the past, as well as in other agricultural crops.

Alternaria alternate and *Alternaria sp.* fungi belonging to the *Alternaria* genus were identified every three years [29]. Only *Alternaria helianthi* species were observed in sunflower replanted fields. Fungi of the genus *Alternaria* cause spot diseases in sunflowers (Figure 4).

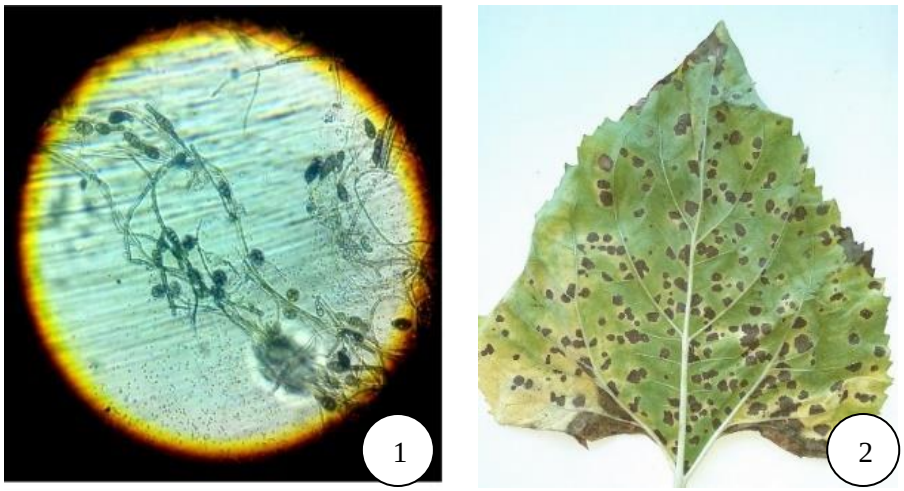

Figure 4. Spot diseases in a sunflower: *1- microscopic vies of Alternaria sp.conidia; 2- sunflower leaf infected by Alternatia leaf spot disease*

In sunflowers, the fungi belonging to the genus *Alternaria* cause leaf spot, and this disease affects adversely the yield of sunflowers. Symptoms of the disease often appear 20–25 days after flowering. Alternaria leaf spot disease infects the leaves, stems and head of the sunflower. But the greatest damage of the disease is observed in the heads, that is, the quantity and quality of seeds decrease.

Alternaria helianthi has been reported to be a major disease in Central Europe, India, Australia, South America and in the humid regions Africa. Yield loss in these areas can range from 15% to 90% and oil loss from 20% to 30% [3]. Yield loss due to this disease has been reported to be from 27% to 80% in India [6, 30].

In addition, *Aspergillus nigerv.Tiegh.* and *Penicillium glaucum Link.* species from secondary parasites that do not directly affect sunflowers were identified in sunflowers. These species were found mostly in sunflower heads infected with cottony rot or head rot and spilled on the ground (Figure 5).

Figure-5. *Mold formation of secondary parasites in sunflower head*

Consequently, our knowledge of the biology of soil-borne phytopathogenic fungi in sunflower fields is mainly related to modern agriculture. Soil-borne phytopathogenic fungi, such as *Fusarium oxysporum, Verticillium dahliae, Rhizoctonia solani* and *Pythium spp.* have been thoroughly studied because they are common in many plants around the world. For example, *Fusarium* wilt has been well studied in more than 100 different plants [2, 24].

The fungi of Fusarium genus are distributed throughout the world and contain at least 300 phylogenetically diverse complexes [25]. This group of ascomycetes is one of the most economically adverse plant pathogens in the world that cause diseases in almost all plants of economic importance and also cause billions of dollars in damage to agriculture each year [4, 10].

4. Conclusions

As a result of our research on soil-borne phytopathogenic fungi in sunflower fields, it became clear that the amount of phytopathogens in sunflower replanted fields differs sharply from that in the fields with crop rotation. That is, most soil phytopathogens are stored in the damaged plant residues or survive in the soil. Therefore, in order to protect sunflower plants from diseases, it is necessary to pay special

attention to crop rotation in the cultivation of sunflower and removal of plant debris from the field after the harvest. This measure prevents the storage of phytopathogenic fungi in the soil. Furthermore, as a result of our research we found out that wheat and maize are good rotation crops for sunflower.

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