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SOIL FIELD ANALYSIS OF SOYBEAN PATHOGENIC FUNGI

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A B S T R A C T

The main objective of this study is the isolation and morphological identification of fungi associated with soybean plants and from soil layers of soybean cultivated fields in Uzbekistan. As a result of a mycological investigation of 160 soybean plant parts sampled from field surveys, 95 saprotrophic and phytopathogenic fungal strains were isolated which have the following distribution according to species assignment: *Alternaria* spp. 3%, *Cercospora kikuchii* 3%, *Mucor* spp. 3%,*Colletotrichum truncatum 3%, Botrytis cinerea 3%, F. Heterosporum 4%, Penissulium* spp. 7%, *Fusarium* spp.8%, *Alternaria alternata* 9%, *Tirichoderma sp 9%, Aspergillus niger* 10%, *Fusarium culmorum* 11%, *Fusarium oxysporum* 13%, *Fusarium solani* 14%. As a result of mycological investigation of soil samples, total of 40 fungal isolates were recovered which have the following species assignment: *Alternaria* sp., *Fusarium* spp., *Trichoderma* sp. *Fusarium oxysporum*, *Fusarium culmorum*, *Alternaria alternata, Fusarium solani*, *Aspergillus niger, Penissulium* sp. *Mucor* sp. The fungal isolates obtained in this study can be used to ease the development of the effective integrated management of soybean diseases in Uzbekistan.

Keywords: Soybean, mycological investigation, fungi, pathogen, isolate, species.

INTRODUCTION

Soybean is a good source of proteins and oil globally. Cultivated soybean (*Glycine max* L.) belongs to the legume family. It is the most widespread crop on our planet, cultivated in more than 60 countries with temperate, subtropical and tropical climates. In developed countries, legumes occupy 10-12% of the area of field crop rotations. Soybean is an unconventional crop in Uzbekistan, and in 2005 it has been introduced into local agriculture. Adaptation of foreign soybean varieties to the agroclimatic conditions of Uzbekistan has been made and also local soybean varieties have been created for cultivation in the republic (Maphosa and Victoria, 2017; Cecilia *et al.,* 2020). The government of Uzbekistan issued a special resolution "On measures to further increase the volume of soybean cultivation in the republic" and approved a “road map” aimed at expanding the cultivation of soybean in the republic. in 2018-2020 According to the decree, 18,500 hectares were allocated for soybean planting in Uzbekistan. Increasing demand of soybean increases the area of production, a trend that is expected to continue in the coming years.

Soybean has a high nutritional value that provides proteins with essential amino acids, complex carbohydrates, minerals and vitamins (Jensen *et al.,* 2012). Due to its high protein content, when included in other meals, it may help reduce malnutrition in children and lactating mothers, thereby improving nutrition in developing countries.

Soybeans have also been reported to have health-beneficial properties (Lemke *et al.,* 2007). They contain biologically active peptides (BAPs), glycosides, isoflavones and phenolic compounds, phytosterols, saponins, alkaloids, sphingolipids and other phytochemicals with health-promoting activities (Yigezu *et al.,* 2009; Horoszkiewicz-Janka *et al.,* 2013).

In addition, soybeans can fix 44-103 kg of atmospheric

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nitrogen per hectare annually, playing an important role in improving soil fertility and reducing the need to apply large amounts of nitrogen fertilizers (Deneke, 2018).

Crop diversification in Uzbekistan and sustainable land management requires increased efforts to reduce mono-planting practices and the transition to crop rotation and intercropping. Numerous studies and field experiments show that crop rotation and intercropping involving soybean can increase soil fertility and increase farmers' profitability

Soybeans are affected by bacterial, fungal and viral diseases. The most common from them are fungi. More than 100 species of fungi have been found on soybeans. The most common are *Fusarium* (Fusarium root rot*),* Alternaria*,* Phomopsis seed decay (Phomopsis spp.), Ascochyta, Cercospora purple seed stain (*Cercospora kikuchii*); Frogeye leaf spot on seed (*Cercospora sojina*); Anthracnose (Colletotrichum spp.); Downy mildew (*Pernospora manshurica*); and various other fungal pathogens.

Fusarium species have been reported that are associated with root rot of soybean. *F. solani* and *F. oxysporum* are the most frequently species associated with root rot on soybean. Other *Fusarium* species include *F. acuminatum*, *F. chlamydosporum*, *F. compactum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. merismoides*, *F. proliferatum*, *F. pseudograminearum*, *F. semitectum*, *F. subglutinans*, and *F. verticilliodes*. *Fusarium* species are widespread soilborne organisms capable of surviving for long periods of time as chlamydospores and as mycelium in plant residues and in soil *F. solani* root rot and wilt of soybean is an important disease that occurs in many soybean-producing countries (Sampaio *et al*., 2020).

Fusarium belongs to the xylem-invading and phloem-invading pathogens that cause vascular wilt diseases (Clare *et al.,* 2010). Pathogens of the genus *Fusarium* target the vascular system, which consists of xylem vessels, tracheary elements that are involved in transport of water and minerals which are absorbed by the roots to the photosynthetic organs, and phloem elements that transports organic photosynthesis products. Vascular wilt pathogens are among the most destructive plant pathogens that can wipe out entire crops. *Fusarium* fungi can spread very well due to their high flexibility in crop rotation, often maintaining the same level of plant disease in different environmental conditions. The relative dominance and importance of the distribution of species of the *Fusarium* and other fungal pathogens affecting soybeans can vary widely depending on the location of the region, the climate and other factors (Scandiani *et al.,* 2011).

Therefore, the main purpose of this study was isolation and morphological identification of fungi from soybean plants and from soil layers of soybean fields in Uzbekistan.

MATERIALS AND METHODS

All collected plant samples were washed twice in distilled water, followed by surface sterilization by soaking for 1 min in 70% ethanol, 4 min in sodium hypochlorite (NaClO, 0.5%), then washed three times in sterile distilled water. After surface sterilization, samples were cut into 1 cm pieces using a sterile scalpel and transferred aseptically onto plates containing potato dextrose agar (PDA). Then fungal colonies were subsequently subcultured to isolate the monosporal fungal strains.

Nutrient media of the following compositions were used for growing fungal isolates: potato glucose agar (distilled water - 1000 ml, potato - 200 g, glucose - 100 g, agar - 20 g, pH-5.5); potato sucrose agar (potato extract - 1000 ml, sucrose - 40 g, agar - 20 g, pH-5.5); potato dextrose agar (distilled water - 1000 ml, potatoes - 200 g, dextrose - 20 g, agar - 20 g, pH-5.5), Chapek nutrient medium (KNO_3 - 2 g, K_2HPO_4 - 1.0 g, $MgSO_4$ - 0.5 g, KCl - 0.5 g, $FeSO_4$ - 0.001 g, sucrose - 20 g, agar-agar 20 g, distilled water - 1000 ml, pH-6). For this purpose, also liquid Chapek-Dox medium was used: $NaNO_3$ - 3.0 g, K2HPO4 - 1.0 g, KCl - 0.5 g, MgSO4 x $7H_2O$ - 0.5 g, FeSO4 x 7H2O - 0.01 g, $ZnSO_4$ - 0.01 g, $CuSO_4$ - 0.001 g, sucrose - 30.0 g, distilled water - 1000 ml, pH-6.

Carnation Leaf Agar (CLA) medium (KCl - 6 g, clove leaves 3–5 mm long, agar-agar - 20 g, distilled water - 1000 ml, pH-5.5) was used to produce macroconidia in the *Fusarium* species. Agar (SNA) medium (KNO3 - 1 g, KH_2PO4 - 1 g, $MgSO_4$ x $7H_2O$ - 0.5 g, KCl - 0.5 g, glucose - 0.2 g, sucrose - 0.2 g, agar - 20 g, distilled water -1000 ml, pH-5.5.) was used to study the morphological structures of *Fusarium* species mycelium. Methylene blue, crystal violet (methyl violet 10B) and iodine-glycerol were used for the preparation of temporary wet mounts in order to observe and identify reproductive and vegetative structures of fungi under the microscope.

Transient preparations were prepared from the isolates and visualized under the binocular microscope. 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 macroconidia, phialides, microconidia, chlamydospores and the colony color and growth rate (Thomma, 2003; Rhaiem *et al.,* 2012; Mamta *et al.,* 2013).

Figure 1. Infected soybean field of Syrdarya region

Portion of 10 g from each collected soil sample were taken for mycological investigation. Then a 1: 100, 1: 1000, and 1: 10000 solutions were prepared from soil samples by mixing them with distilled and sterilized water in the sterilized 300 ml flasks. One-ml of each solution at a ratio of 1: 10,000 was taken and inoculated in agar (WA). After

Figure 3. Preparation of soil samples for mycological investigation

RESULTS

soybeans from representatives of the legume family (*Leguminosae*) in several fields of Tashkent, Samarkand, Navoi and Syrdarya regions of different geographical zones of Uzbekistan. During the survey, soil samples were taken in special containers at a depth of 10, 20 and 30 cm from soybean fields (Figures 1-2).

Figure 2. Soil samples taken for mycological analysis

inoculation, the fungi were cultured at +25 + 26 °C in an artificial climate chamber for 3-5 days. From day 3-5, fungal colonies were separated according to species composition. The isolates were grown in potato dextrose agar (PDA) nutrient medium in an artificial climate chamber for 15 days at +25 + 26°C (Figures 3-5).

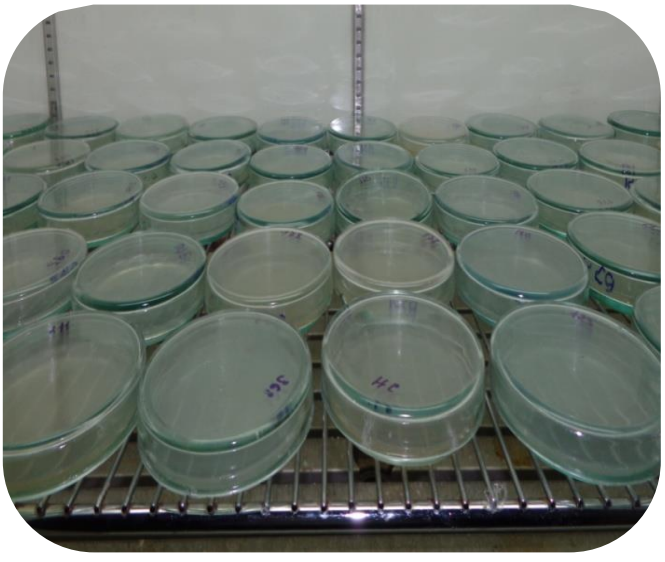

Figure 4. Soil samples in an artificial climate chamber

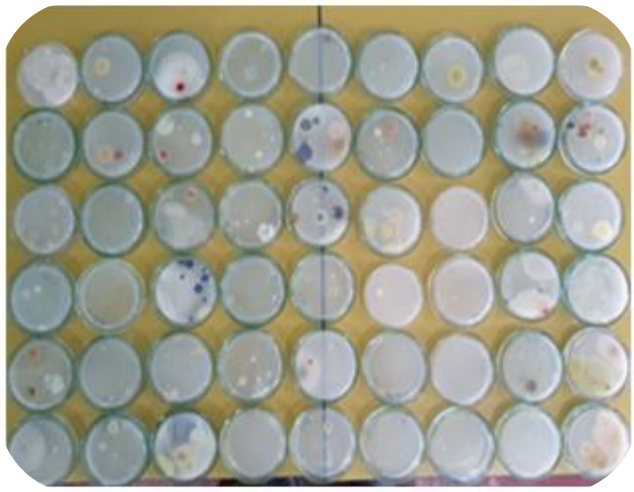

Figure 5. Soil samples after 72 h of incubation in PDA

Mycological investigation of the 1st field soil samples from Samarkand region revealed the presence of *F. oxysporum, F.solani, F. heterosporum* fungi in the soil layers of 10, 20 and 30 cm (Figures 6).

Figure 6. Mycological analysis of soil samples from Samarkand region

Mycological investigation of the 2nd field soil samples from the Navoi region showed the presence of *F. oxysporum, F. solani,* and *F. culmorum* in the soil layers of 10, 20 and 30 cm (Figures 7).

Figure 7. Mycological analysis of soil samples from the Navoi region.

When the 3rd field soil samples from the Tashkent region were mycologically investigated, Trichoderma sp., *Penissulium* sp. and *Aspergillus niger* were found in the soil layers of 10, 20 and 30 cm depth (Figure 8).

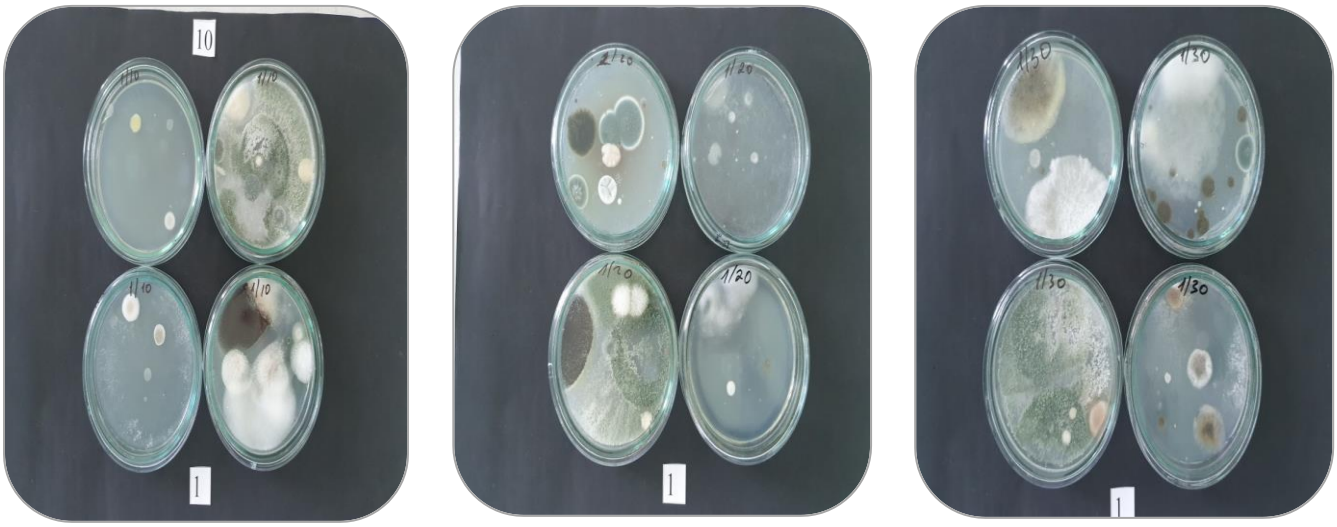

Figure 8. Mycological analysis of soil samples from Tashkent region

As a result of experiments, mainly *F. culmorum*, *F. oxysporum*, *Alternaria alternata, F. solani*, *A. niger, Alternaria* sp.*, Fusarium* spp.*, Trichoderma* sp., *Penicillium* sp., *Mucor* sp. were revealed (Figures 9-11).

F. oxysporum - large conidia were formed in aerial mycelia, sporodochia or pionnots and had a thin cell coat. The shape was filiform-arc-shaped, umbellate, most of the same width, the tip cells are short and regularly bent, well-formed pedicel, 3-5 septate.

Figure 9 Subcultured monosporal fungal strain from Tashkent region

Figure 10 Subcultured monosporal fungal strain from Navoi region

Figure 11 Subcultured monosporal fungal strain from Samarkand region

Aerial mycelium was fluffy, spider web-like, film-like, well-developed, semi-pink, light-purple, pale red, purple, dark purple, sometimes black, sometimes colorless. Chlamydospores were produced in large numbers and form a chain of one or two cells at the end of the mycelium.

The size of large three-barred conidia was 25-30x3.8-4 (-25-40x3.7-5) µm, five-barred conidia is 37-40x3.9-4.3 (30-50x3-5) µm (Figure 12-13).

The macroconidia of *F. oxysporum* and *F. solani* were studied under a binocular microscope by preparing a temporary preparation.

Figure 12. *Fusarium oxysporum*

Figure 13. *Fusarium oxysporum* conidia in KDA nutrient medium (x400 times magnified image)

Large conidia were formed in aerial mycelia, sporodochia or pionnots and have a thin cell coat. The shape was filiform-arc-shaped, umbellate, mostly of the same width, tip cells were short and regularly curved, with a well-formed peduncle at the base, 3-5 septa. It produces numerous small conidia, oval, oblong, unicellular or unicellular, in conidial bands, aerial mycelia, heads and chains.

Colonies of *Alternaria alternata* species were fast-growing, gray, greener, dark gray, dark olive, or black in color. Conidia often form long and branched chains. Conidia were inverted tuft, ovoid or narrow ellipsoidal, gray-brown or olive-brown in color, 20-50x8-12 µm in size in culture, transverse septate, 1-2 longitudinal septate in 1 or several transverse segments. Secondary conidiophores are short, apical (usually up to 5-10 µm in length, rarely up to 35 µm), sometimes lateral. In KSA and V-4 media, the amount of aerial mycelium is moderate, colorless to light brown, with a gray tint when conidia were abundant. In the natural nutrient environment, the aerial mycelium was dense, colorless, yellowish, light-colored, less often light-gray. Sporulation usually begins between 5–10-day old colonies. The sporulation habit of isolates of this group of species is very variable. Chains of conidia are short or long, few or strongly branched. [Figures 14-15)

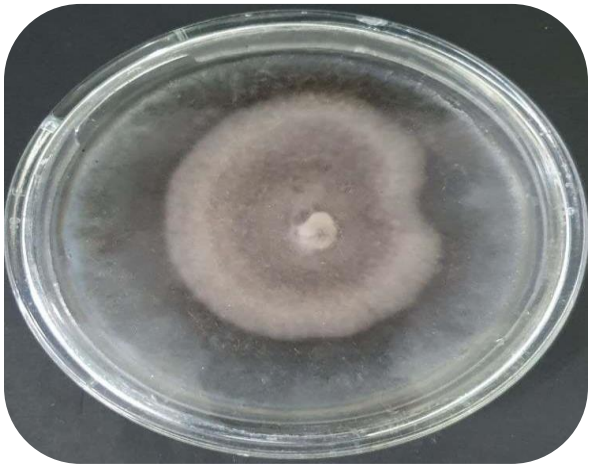

Figure 14. *Alternaria alternata*

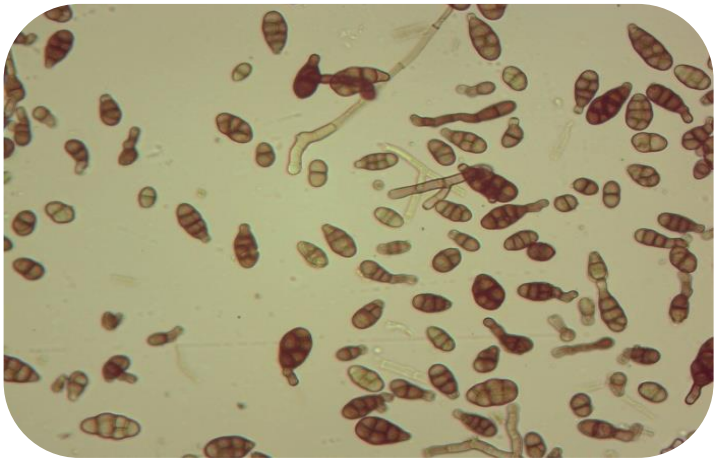

Figure 15. *Alternaria alternata* conidia (x400 times magnified image)

In the irrigated fields of the districts of Tashkent, Syrdarya, Navoi and Samarkand regions of our republic, the fields planted with soybean plants representing the Leguminosae family were subjected to phytosanitary control. It was observed that there are disease symptoms in the plant organs, i.e., root rot, root neck rot, leaf spotting, stem wilting and various spots.

Figure 16. Investigated soybean field of Syrdarya region

As a result of the observations, samples were taken from various organs of these affected plants and herbariums were brought to the laboratory for mycological examination in order to isolate phytopathogenic mycomycetes. (Figure 17)

Figure 17. Soybean plants infected with fusarium wilt

To separate the isolated phytopathogenic fungi into separate species, as well as to determine their species composition, to determine the structure of their macro- and microconidia, they were planted in separate nutrient media and grown for 5 days (Figure 18).

a) b) c)

Figure 18. a) samples of fungal colonies grown for 1 day. b) samples of fungal colonies grown for 5 days c) separated monosporal fungal colonies

As a result of mycological investigation of 160 plant parts that were collected during field surveys (Figure 19), 95 fungal strains were isolated which have the following distribution according to species assignment: *Alternaria* sp. 3%, *A. niger* 10%, *Fusarium* spp. 8%,*A. alternata* 9%, *F. culmorum* 11%, *F. oxysporum* 13%, *F. solani* 14%, *Mucor* sp. 3%, *Penissulium* sp. 7%, *Cercospora kikuchii* 3%, *Colletotrichum truncatum* 3% *Botrytis cinerea* 3%, *Trichoderma* sp. – 9%, *F. Heterosporum* -4% (Figure 19). Isolated fungal strains belonged to 14 species from 10 genera.

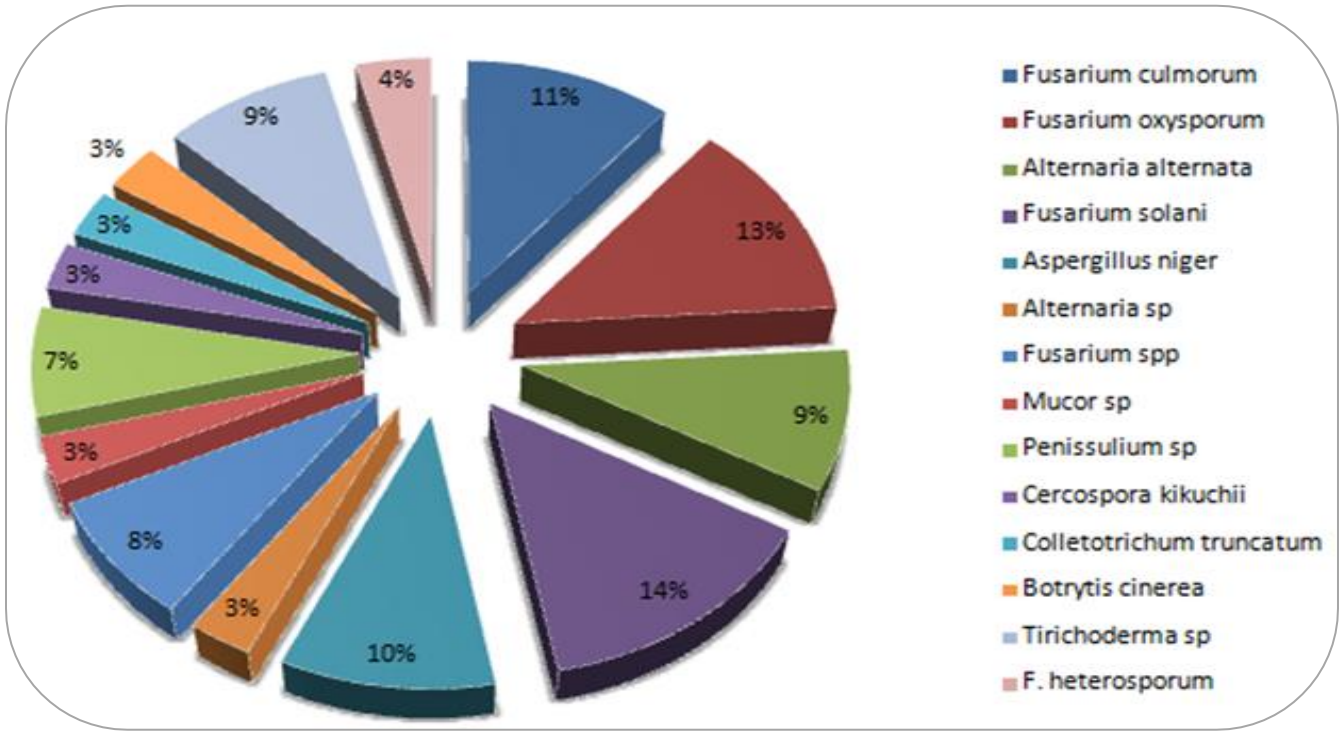

Figure 19. Distribution of fungal species isolated from soybean plant samples, %.

Figure 20. *F. solani* fungus (in KDA nutrient medium)

F. solani conidia (in KDA nutrient medium) magnification x 400 times

Results of this study showed that the most common species of fungi isolated from soybean plants in Uzbekistan were *F. solani, F. oxysporum, F. culmorum, A. niger* followed by Trichoderma sp., Penissulium sp., *A. alternata*, *Cercospora kikuchii* and others.

DISCUSSION

About 200 phytopathogenic microorganisms (primarily fungi) have been found on soybean (Hartman *et al.,* 2015), and it is reported that about 30 species can cause significant economic damage (Roy *et al.,* 2000). Mostly lives in soybean seed and thus spread and distribute in other soybean-growing regions worldwide (Backman *et al.,* 1985; Pioli *et al.,* 1999, Vidić *et al.,* 2006). This article describes the analysis of mycological studies carried out on fields sown with soybeans in different geographical zones of Uzbekistan. Some areas of the Tashkent, Samarkand, Navoi and Syrdarya regions of the Republic of Uzbekistan, a phytosanitary examination was carried out on soybean fields for the collection of soil and disease samples. Collected samples were examined in laboratory for the isolation of phytopathogenic fungi species. It was identified on the basis of their morphological, systematic, biological characteristics. According to Marin-Menguiano *et al.* (2019), fungal diseases have been increasing day by day in many crops that affects both its quality and quantity and they have become an important bottleneck for the development of sustainable agricultural.

Results of this study showed that the isolated strains *F. solani, F. oxysporum, F. culmorum, Aspergillus niger*, *Alternaria alternata sp, Fusarium* spp. *and Trichoderma* sp. were present in high percentages in members of the *Leguminosae* family. Infected seedling of soybean moderate and acute darkening of the epicotyl or roots was noticed. The findings of present research are according with study performed by Ruzmetov *et al.* (2021), in which, the frequency of *F. solani, A. alternate, A. niger, Ascohita rabiei, F. culmorum* and *F.oxysporum* fungi isolated from legumes was found to be high. Following species of fungi genera: Diaporthe, Fusarium and Alternaria as well as species: *Cercospora kikuchii*, *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum*, *Botrytis cinerea* Persoon, *Macrophomina phaseolina*, and *Peronospora manshurica* are the most common fungi of soybean seed globally (Rather *et al.,* 2010: Tenuta *et al.,* 2015).

A. niger is a filamentous fungus of ascomycetes that is ubiquitous in the environment. This fungus is being responsible for postharvest decay of grains worldwide and it was shown that increase of environmental temperature because of global warming, especially the increasing frequency and duration of heat-wave during summer periods can result in an increased threat to food safety by stimulating growth of *A. niger* (Csernus *et al.,* 2013; Sherimbetov *et al.,* 2020).

As a result of this experiment, it was determined that saprotrophic and phytopathogenic fungal strains belong to 10 genera and 14 species. According to the classification of Leslie and Summarell (2006) there are 70 species of fungi in the Fusarium genus, while in the conditions of the Republic of Uzbekistan, it was determined that there are 17 species of this genus. *Alternaria* species (especially the sense lato type called *A. alternata*) have been found to cause diseases in leaves, stems and pods of soybean and chickpea in natural conditions (Khasanov *et al.,* 2000; Khasanov *et al.,* 2013). In our republic, 2 types of Alternaria species, strains of *A. alternat*a, *A. tenuissima* species, which cause black sunken spot disease, were detected in the leaves of soybean, chickpea, mung bean and bean plants (Sherimbetov *et al.,* 2020).

CONCLUSION

During mycological investigation of plant samples collected from soybean fields of the Republic of Uzbekistan, 95 fungal strains were isolated and assigned to 14 species from 10 genera. As a result of mycological investigation of soil samples total of 40 fungal isolates were recovered and assigned according to the species classification. The most common fungal species isolated from soybean plants in Uzbekistan, were *F. solani, F. oxysporum, F. culmorum, A. niger* followed by *Trichoderma* sp., *Penissulium* sp., *A. alternata, C. kikuchii* and others.

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Contribution of Authors:

Matniyazova K. Xudayberganovna	:	Conduct trials and wrote manuscript
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Yuldashov U. Khayitovich	:	Prepared tables, figures and graphs