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Identification of sunflower seed pathogens and their effect on seed germination against the background of plant damage by *Septoria* leaf spot

Kristina Levitskaya[1]*, Viktor Lyakh[1,2], Oksana Afanasieva[3]
[1]Institute of Oilseed Crops of NAAS, 1 Institutskaya St , Zaporozhye 70417, Ukraine, [2]Zaporozhye National University, 66 Zhukovsky St, Zaporozhye 69600, Ukraine,
[3]Institute of Plant Protection of NAAS, 33 Vasylkivska St, Kyiv 03022, Ukraine
*E-mail: *krlevitskaya92@gmail.com*

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Abstract

Many pathogens spread with sunflower seeds, which later cause plant diseases. The aim of our work was to isolate and identify sunflower seed pathogens and to investigate their effect on the germination of seeds that were formed on plants with different degrees of damage by *Septoria* leaf spot. Field germination of seeds was analyzed on a stationary artificial infectious background in 2020–2021, and laboratory germination and phytopathological analysis – out in laboratory conditions in 2021. All four experimental samples were found to be infected with seed infection, and the level of pathogen infection ranged from 5% to 75%. The isolated pathogens belonged to the genera *Alternaria, Aspergillus, Cladosporium, Mucor, Rhizopus, Trichoderma* and *Penicillium*. A bacterial infection was also detected. Most pathogens were found on seeds that were formed on plants affected by *Septoria* leaf spot. The main pathogens here were representatives of the genera *Alternaria* with infection from 45% to 75%, *Cladosporim* – 10% and bacterial infection – from 15% to 25%. The smallest number of pathogens was isolated on the seeds of healthy plants. Seed samples, both in the field and in the laboratory, differed significantly in terms of germination. It was found that the highest level of seed infection with various pathogens was characteristic of samples with low germination. The presence of the *Alternaria* pathogen had a negative effect on seed germination. No infection of sunflower seeds with *Septoria* leaf spot was detected, despite the high level of damage by this disease to the plants on which it was formed.

Key words: sunflower, *Septoria* disease, seed-borne pathogens, seeds germination, moist-chamber, nutrient medium.

INTRODUCTION

Healthy seeds are the most important condition for the quality of food crops. Seeds are one of the main factors in the spread of pathogens and are of primary importance for understanding the epidemiology of plant diseases (Elmer, 2001; Majumder et al., 2013). Seed-borne pathogens have a particular epidemiological advantage for long-distance dispersal. Seed dispersal of the pathogen increases its chances of survival and establishing contact with a susceptible host (Elmer, 2001).

Many fungal pathogens leave spores on the seed coat. The same thing happens with mycelium. Further, reproduction can occur in already infected tissues of the host (Guan et al., 2020).

The infection can persist in the seed and can be a significant risk as a source of inoculum (Elmer, 2001; Turkington et al., 2002; Barret et al., 2016).

Seed-borne fungal pathogens affect the growth and productivity of agricultural plants. They lead to necrosis and rotting of seeds, discoloration, reduced germination, and damage to plant seedlings (Barret et al., 2016; Goko et al., 2021).

Early detection and identification of infected seed lots can slow the spread of pathogens and isolate new disease outbreaks (Elmer, 2001).

Sunflower (*Helianthus annuus* L.) is one of the leading crops grown for oil production. Sunflower seeds contain more than 40% edible oil and 23% proteins. Sunflower is a good source of fiber, vitamin E, copper, zinc and vitamin B complex (Addrah et al., 2020).

Pathogens transmitted with seeds lead to a decrease in sunflower yield. Also, they affect the decrease in the content of proteins, carbohydrates, cholesterol, iodine and can cause an increase in acidity (Irum, 2009). In his research, Asim et al. (2008) identified fungal pathogens such as *Aspergillus niger*, *A. flaves*, *Alternaria zinniae*, *Cladosporium oxysporum*, *Curvularia* sp., *Drecheslera* sp., *Macrophomina phasiolina*, *Penecillium* sp., *Phoma* sp., *Rhizopus* sp..

Septoria leaf spot is a harmful disease of sunflower plants, which leads to reduced growth, chlorophyll content in the leaves and premature drying of the leaves. Under favorable weather conditions, this disease leads to significant losses yield (Brand et al., 2018). The pathogen of sunflower *Septoria* disease is the fungus *Septoria helianthi* Ellis & Kellerm. *Septoria* leaf spot is widespread in Asia, Africa, North and South America, and Australia (Irum, 2009). The transmission of the pathogen *S. helianthi* by sunflower seeds has not been researched enough. Some scientists believe that the pathogen can spread through seeds, while others argue that it can not.

Identification and characterization of seed-borne pathogens is an important part of comprehensive prevention and response strategies designed to protect against seed-borne diseases (Guan et al., 2020).

It can be quite difficult to obtain material from contamination of seeds, because many species fungal do not fruit on a regular, but only under specific conditions. To improve infection detection rates, mycologists have developed a laboratory method to increase detection, the moist-chamber technique. This technique involves keeping the substratum moist over long time in a chamber in which environmental conditions may be manipulated (Krug, 2004). Culture media are used to culture microorganisms in the laboratory to provide the nutrients needed for their growth and maintenance also often (Uthayasooriyan et al., 2016).

The aim of the research was to isolate and identify seed-borne pathogens of sunflower and to study their influence on the germination of seeds formed on plants with varying degrees of *Septoria* infection.

MATERIALS AND METHODS

Field studies on the assessment of plant resistance to *Septoria* leaf spot and the analysis of field germination of seeds were carried out on a stationary artificial infectious plot of the Institute of Oilseed Crops of the National Academy of Agrarian Sciences of Ukraine during 2019–2021. The stationary infectious plot was established in the field crop rotation of the Institute of Oilseed Crops in 2005 to evaluate the breeding material of oilseeds for a complex of diseases. The weather conditions of the growing season were favorable during the research years for the development of sunflower pathogens (Levitskaya et al., 2023).

Seeds (samples 1–4) obtained from self-pollination of the F1 hybrid of the combination of crossing ZL58A x HA-R7 with varying degrees of plant damage by *Septoria* leaf spot served as the material for the study. Plant F1, which formed the seeds of the 1st sample, was not affected by *Septoria* leaf spot. The seeds of samples 2, 3 and 4 were obtained from F1 plants with an average degree of infection with this disease, that is, the hybrid plants had symptoms of *Septoria* leaf spot on the lower and middle leaves. The assessment of the severity of plant F1 damage was carried out in 2019 by visual inspection of all leaves against a scale modified by according to base scale "Scale for estimating wheat resistance to *Septoria* pathogen (*S. tritici*)" (Levitskaya & Lyakh, 2022).

To assess the field germination, seeds of test samples were sown by 200 seeds in 2020 and by 120-160 seeds in 2021 on 3–4 row plots according to the sowing scheme 35 x 70.

The laboratory germination of sunflower seeds was evaluated according to DSTU 4138-2002 (DSTU 4138, 2002). The seeds of each sample were planted in equal quantities in Petri dishes on filter paper. Two discs of paper were placed on the bottom of each cup, and one disc was placed on the lid and moistened to full moisture content. The seeds

were germinated in the dark at a temperature of 20°C in a thermostat. Growth energy was noted on day 4, and germination on day 10.

Phytopathological examination of sunflower seeds was carried out according to DSTU 4138-2002 in the laboratory of phytopathology of the Institute of Plant Protection of NAAS. In order to determine the damage to seeds of phytopathogens, it was placed on a moist-chamber and a sterile (PGA) nutrient medium. Pathogens were identified according to the methods of Belay (1982) (Pidoplichko, 1977; DSTU 4138, 2002).

To place the seeds on the nutrient medium, it was previously washed with running water and sterilized with a 96% alcohol solution. Under sterile conditions, superficially disinfected sunflower seeds were placed in Petri dishes with previously poured potato-glucose medium (PGA) with the addition of Triton-X 100 and the antibiotic Gentamicin. Inspection of Petri dishes in moist-chamber conditions was carried out on day 7–10, on day 10–14 in PGA. Identification of phytopathogens was carried out using binoculars and a microscope based on the morphological characteristics of conidia of phytopathogens. For each sample, 20 seeds were analyzed both in the moist-chamber and on the nutrient medium.

Statistical processing of the obtained data was carried out using the Microsoft Excel application program package (Kronthaler, 2023).

The percentage error was determined by the formula (1):

$$S_p = \sqrt{\frac{P \times (100 - P)}{n}},$$

where Sp is the percentage error; P is the percentage of affected plants; n – the total number of analyzed plants (Rokitsky, 1973)

Table 1. Pathogen infection of seeds obtained from F_1 sunflower plants with different susceptibility to *Septoria* leaf spot, 2021

Sample	Damage to F_1 plants by *Septoria* leaf spot in 2019	Number of infected seeds in F_2 (%)			
		superficial infection (the moist-chamber)		internal infection (the sterile nutrient medium)	
1	Not damage	*Alternaria*	15	*Penicillium*	5
		Aspergillus	5	*Trichoderma*	50
		Rhizopus	25	-	-
	Total damage (%)		45		55
2	an average degree of infection (affected leaves of the lower and middle layers)	*Alternaria*	75	*Alternaria*	15
		Bacterial infection	25	Bacterial infection	25
		Cladosporium	10	-	-
				-	-
		Mucor	50	-	-
		Penicillium	5	*Penicillium*	10
	Total damage (%)		> 100		50
3	an average degree of infection (affected leaves of the lower and middle layers)	*Alternaria*	45	*Penicillium*	15
		Bacterial infection	15	*Trichoderma*	5
		Cladosporium	10	-	-
		Rhizopus	40	*Rhizopus*	15
	Total damage (%)		> 100		35
4	an average degree of infection (affected leaves of the lower and middle layers)	*Alternaria*	20	-	-
		Aspergillus	35	-	-
		Bacterial infection	5	-	-
		Mucor	15	-	-
	Total damage (%)		75	not revealed	

RESULTS AND DISCUSSION

Analysis of the experimental seeds revealed the presence of fungi belonging to the genera *Alternaria, Aspergillus, Cladosporium, Mucor, Rhizopus, Trichoderma, Penicillium* and a bacterial infection (Table 1).

All four experimental samples had seed infection (Figure 1, 2). The level of infection with a single pathogen ranged from 5% to 75%.

The seeds of samples 2 and 3, which were formed in 2019 on plants affected by *Septoria* leaf spot on the lower and middle layers of the leaves, turned out to be the most infected. In the moist-chamber conditions, six pathogens were isolated from the seeds of samples 2 and 3. Among them, representatives of the genera *Alternaria* with seed infection from 45% to 75%, *Cladosporim* - 10%, and bacterial infection from 15% to 25% were distributed in both samples. In addition, pathogens of the genera *Mucor* and

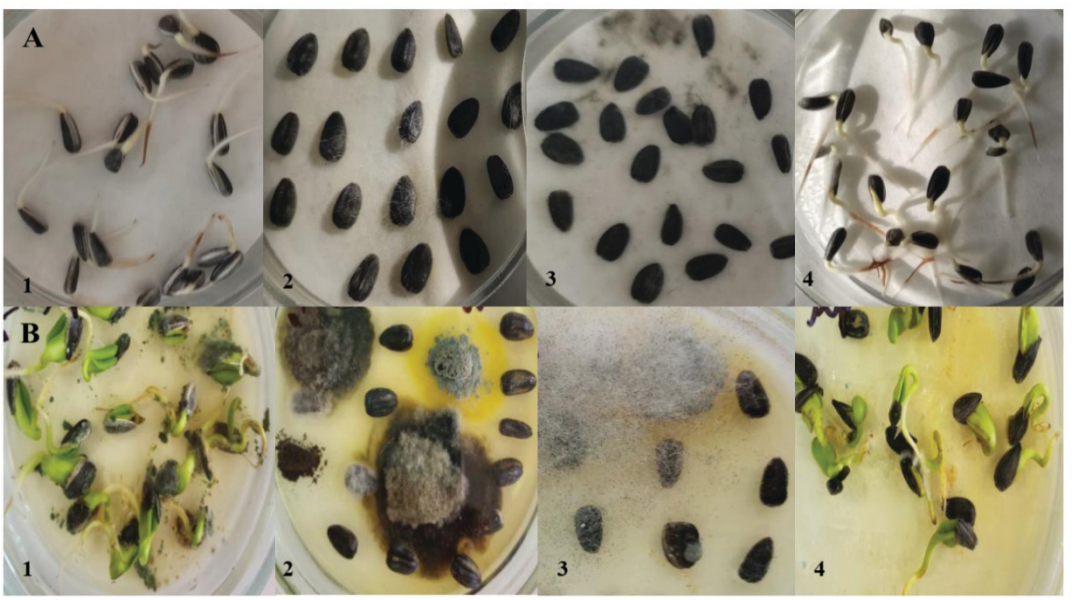

Figure 1. Seeds of sunflower samples 1–4 in a moist-chamber (A) and on a nutrient medium (B)

Figure 2. Seeds of sunflower samples 2 and 3 contamination by pathogens in a moist-chamber

Penicillium were isolated from the seeds of sample 2 with an infection level of 5% to 50%, and in sample 3 Rhizopus was present with an infection level of 15% to 40%. On the nutrient medium, pathogens of the genus *Alternaria* were isolated from the seeds of sample 2 with an infection level of 15%, bacterial infection – 25%. Under these conditions, *Penicillium* was also isolated in both samples with a contamination level of 10% to 15%.

The seeds of sample 1 in 2019 were formed on a plant that was characterized by a complete absence of Septoria leaf spot damage. In the conditions of a humid chamber, pathogens of the genus *Alternaria* were isolated from the seeds of this sample, with an infection level of 15%, *Aspergillus* – 5%, and *Rhizopus* – 40%. *Penicillium*, infection level 10%, and *Trichoderma* – 50% were isolated from seeds on a nutrient medium.

Seed infection in sample 4 was isolated only using a moist-chamber. Among the isolated pathogens were *Aspergillus* with an infection rate of 35%, *Alternaria* – 20%, bacterial infection – 5% and *Mucor* – 15%.

It was found that *Alternaria* was the most common seed pathogen and was present on the seeds of all four experimental samples. However, samples 2 and 3 (45% and 75%) had the highest level of *Alternaria* infection. For samples 2 and 3, unlike the others, *Cladosporium* damage was also characteristic. Bacterial infection was observed in three *Septoria*-susceptible samples, but samples 2 and 3 were more affected.

It should be noted that representatives of the genera *Alternaria, Aspergillus, Cladosporium, Mucor, Penicillium* and *Rhizopus* are pathogenic among the selected fungi. In addition, pathogens of the genera *Alternaria* and *Aspergillus* can release mycotoxins.

In general, the greatest diversity of pathogens and the greatest level of infection were found in samples that were formed on plants susceptible to *Septoria* leaf spot. This may be due to the effect of *S. helianthi* on reducing the immunity of plants and their resistance to infection.

However, we were unable to isolate the pathogen *S. helianthi* from the seeds. Perhaps this is due to the presence of other pathogens, which developed much faster both in the moist-chamber and on the nutrient medium than this pathogen, thereby inhibiting its further development.

Data on the slow development of *S. helianthi* in pure culture are described in the literature. Thus, it was found that in the presence of pathogens that avoided surface sterilization, the mycelium of the *Septoria* fungus usually grew quite slowly. Subsequently, it was difficult to obtain it in pure culture (Beach, 1918).

Another reason for the absence of this pathogen on sunflower seeds may be that it belongs to systemic fungi, like other *Septoria* pathogens – *S. linicola, S. glycines*. After all, it has been established that systemic pathogens penetrate the cells of the ovary with an infectious mycelium and produce teliospores or "sleeping spores". Such spores are not isolated during seed analysis and remain dormant on the surface until the seed germinates. Next, plants are infected during germination and the fungus develops as the seedling develops (Nallathambi et al., 2020).

Table 2 shows data on the field and laboratory seed germination of experimental samples 1–4. Differences between the samples in seed germination were found in both experiments (Table 2).

It can be seen from Table 2 that the seeds of sample 1, which were formed on a plant not affected by

Table 2. Field and laboratory germination of the seeds obtained from plants with different levels of *Septoria* infection

Sample	In the field				In vitro	
	sown seeds, total (pcs)		of them germinated plants (%)		not germinated (%)	germinated (%)
	2020	2021	2020	2021		
1	200	160	81.5±2.7	71.3±3.6	0	100
2	200	120	16.5±2.6	5.0±2.0	100	0
3	200	120	10.5±2.2	8.3±2.5	100	0
4	200	160	78.5±2.9	75.6±3.4	0	100

Septotia leaf spot, were characterized by sufficiently high germination in field conditions. It varied over the years from 71.2% to 81.5%. In laboratory conditions, the seeds of this sample germinated 100%.

Field germination of seeds in samples 2 and 3 was low in both years of research. In 2020, it did not exceed 20%, and in 2021 – 10%. In the laboratory conditions of 2021, this seed did not germinate at all, which is clearly visible not only from Table 2, but also from Figure 1A and 2 when the seed was planted in a moist-chamber. At the same time, it should be remembered that the mother plants that formed the seeds of these samples in 2019 had *Septoria* leaf spot-affected leaves of the lower and middle layers.

At the same time, the seeds of sample 4 with an average degree of damage to the mother plant *S. helianthi* in 2019, as well as samples 2 and 3, in the field conditions of 2020 and 2021 were characterized by a similarity that was not inferior to the field similarity of sample 1. Under laboratory conditions, 100 % of the seeds of sample 4 were also germinated.

So, from the obtained data, it can be seen that the seeds of samples 2 and 3, both in field and laboratory conditions, differed significantly in terms of seed germination from the other two samples. Apparently, the poor germination of seeds in these samples was caused by seed infection against the background of damage to the mother plants by *Septoria* leaf spot. These samples were characterized by the greatest variety of pathogens and the highest level of seed contamination. Samples 1 and 4, in comparison with samples 2 and 3, had a significantly lower level of seed infection, which was reflected in its field and laboratory germination.

So, our study showed that the sunflower seed coat is heavily contaminated with various pathogens, which contributes to the rapid and widespread spread of diseases. In addition, pathogens can have a significant impact on seed germination.

It is believed that the pathogen can contaminate the surface of the seed or it can be infected systemically from the mother plant. During systemic infections, the fungus germinates into an embryo and later develops from a seed into a seedling (Flett, 2017).

On sunflower seeds, scientists have found various species of fungi belonging to the genera *Alternaria, Aspergillus, Cladosporium, Curvularia, Drechslera, Fusarium, Penicillium* and the pathogen *Phomopsis macdonaldii* (Irum, 2009). Among them, the common species are *Alternaria alternata, A. helianthi, Fusarium oxysporum, F. solani, Penicillium expansum, P. brevicompactum, Aspergillus niger, A. flavus, A. fumigatus, A. terreus, Rhizopus stolonifer, Mucor hiemalis* (Abdullah & Al-Mosawi, 2010). It was reported that the fungus Plasmopara halstedii is spread by sunflower seeds. Mycelia of this pathogen were found in sunflower seeds in all tissues of the flower, including sepals, petals, pistil and stamens (Cohen & Sackston, 1974).

Fungi of the genus *Alternaria* were also isolated from sunflower seeds. In the case of completely contaminated seeds on seed coats, embryos, and cotyledons, infected by representatives of this genus varied from 32% to 91% (Kgatle et al., 2018). Addrah et al. (2020) have also detected pathogens of the genus *Alternaria* such as *A. helianthi* and *A. alternata* on sunflower seeds. They note that the coats of sunflower seeds are the main carriers of pathogens. The transmission of the pathogen *Alternaria helianthi* during seeding and growth of sunflower seedlings was investigated by Lakshmi Prasad et al. (2010).

In our studies, seeds of experimental samples were affected by fungal pathogens belonging to 7 genera - *Alternaria, Aspergillus, Cladosporium, Mucor, Penicillium, Rhizopus, Trichoderma*, as well as bacterial infection. More over, most pathogens were localized on the seed coat, which is consistent with the data of other researchers.

Analyzing the level of infection of seeds by various pathogens and comparing it with germination, it can be stated that fungi of the genus *Alternaria* had the greatest negative impact on the quality of sunflower seeds. This effect has been repeatedly highlighted in the literature. Thus, in the studies of Kgatle et al. (2018), sunflower seeds that were heavily infected with A. helianthi showed low germination and a high rate of seedling decay. Asim Mohamed El Siddig El Azhary (2008) stated a decrease in germination of sunflower plants with seeds infected with *Alternaria* infection.

It is also known that various species of Aspergillus can negatively affect seed germination and seed quality by producing aflatoxins (Lakshmi et al., 2010). However, in our studies, samples with low seed germination were not affected by this pathogen at all.

Regarding the transmission of *S. helianthi* by sunflower seeds, the information is quite contradictory. According to Xie-Jing L & Bao-Nan L (1988), sunflower seeds do not contain this pathogen. However, André Stéubli allows the possibility of the disease spreading by seeds due to the availability of data on the spread of infection with seeds of other crops (Gindrat & Freil, 1997). According to Sutruedee Prathuangwong et al. (1989), seeds infected with *S. helianthi* were the main source of infection. Tests were carried out on the isolation of the pathogen from seeds, which showed that the pathogen was unlikely to be carried by seeds (Shavi, 1951). Our studies also did not detect *Septoria* pathogen in sunflower seeds, despite the fact that the plants that formed the experimental seeds had a significant level of infection.

However, according to literature sources, *Septoria* pathogens that affect other plant species can be transmitted with seeds. Thus, it is known about the spread of *Septoria* leaf spot with wheat, parsley, and celery seeds (Mauder, 1964; Shah et al., 2002; Majumder et al., 2013; Tok & Kurt, 2019). It is noted that infected wheat seeds were of poor germination (Cunfer & Johnson, 1981). A decrease in seed germination was observed even in laboratory experiments (Shah et al., 2002).

CONCLUSIONS

It was established that under the conditions of the artificial infectious nursery of the Institute of Oilseed Crops of NAAS, sunflower seeds were affected by pathogens belonging to 7 genera of fungi – *Alternaria, Aspergillus, Cladosporium, Mucor, Penicillium, Rhizopus, Trichoderma*, as well as bacterial infection. Among the isolated fungi, only *Trichoderma* representatives are not harmful. In most of the seeds, only the coat was infected.

No surface or systemic infection of sunflower seeds by *Septoria* pathogen was found, despite the high level of infection with this disease of the plants that formed these seeds.

The highest level of infection with a total damage of more than 100% and the greatest variety of pathogens were found on non-germinating seeds that were formed on plants affected by *Septoria* leaf spot.

The relationship between the level of seed infection with the *Alternaria* pathogen and the ability of seeds to germinate was revealed. The negative impact of this pathogen affects both field and laboratory seed germination. It has been established that the *Alternaria* pathogen can be localized both outside and inside the seed.

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