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RESEARCH ON MICROBIOTA ASSOCIATED WITH SUNFLOWER SEEDS

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Abstract

The aim of our research has been to detect and identify the microbiota associated with sunflower seeds. Biological material was represented by seeds samples from romanian hybrids Suria CL and Demetra and from foreign hybrids ES Belfis CLP, LG5077, QC Bravo, P64LE 99, P64LE 162 and P64LE 280. Pathogens from colonies developed after six days were identified, and their incidence was calculated for both untreated seeds and treated seeds with Lumisena 200 FS. In untreated seeds samples fungi from the genera Alternaria, Aspergillus, and Rhizopus were detected as well as bacterial colonies. Alternaria spp. recorded the highest incidence on ES Belfis CLP hybrid (50%), followed by Demetra (30%). Colonies of Aspergillus spp. were detected with an incidence of 33.3% for P64LE 162 hybrid. On treated seeds, Alternaria fungi recorded 20% incidence for Demetra hybrid, and 3.3% for P64LE99 hybrid, while Penicillium spp. showed an incidence of 3.3% for Demetra hybrid. Therefore, the antifungal effect of the tested product has been observed.

Key words*: sunflower, hybrid, seeds, microbiota.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseeds crops globally, being extensively cultivated in numerous countries, including Ukraine, the Russian Federation, Argentina, China, Romania, Turkey, Bulgaria, France, Hungary, Tanzania, Kazakhstan, Moldova, the United States of America, Spain, South Africa, Serbia, Italy, Uganda, and India. Romania plays a significant role in the international market for this crop due to its considerable production (Chiurciu et al., 2023). Sunflowers are grown primarily for their seeds, which contain 40-50% oil and 17-20% protein (Haj Sghaier et al., 2023).

Seeds harbor diverse microbial groups which play a significant role in maintaining seed vigor, enhancing germination and modulating plant growth (Vannier et al., 2018; Kumar et al., 2024).

The microbial community associated with seeds depends on plant species, seed development, geographical locations, and the presence of other plant pathogens (Houlden et al., 2008; Nelson, 2018).

Pathogens transmitted through seeds pose a significant problem in sunflower cultivation, leading to reduced seed viability and germination as well as seedling vigour, poor stand of the crop in the field and low yields (Afzal et al., 2010). Additionally, their presence affects the biochemical composition of the seeds, including the content of proteins, carbohydrates, and lipids, thus influencing their quality (Mukhtar, 2009).

Among the microorganisms that interact with seeds during storage, fungi play a dominant role in reducing their quality and longevity (Ivan & Cristea, 2015; Cristea et al., 2009).

Studies conducted by Asim et al. (2008) have highlighted the presence of fungi such as *Aspergillus niger, A. flavus, Alternaria zinniae, Cladosporium oxysporum, Curvularia* sp., *Drechslera* sp., *Macrophomina phaseolina, Penicillium* sp., *Phoma* sp., and *Rhizopus* sp. Also, other studies highlight the presence of fungal isolates belonging to the genera

Alternaria, *Aspergillus*, *Cladosporium*, *Mucor*, *Rhizopus*, *Trichoderma* and *Penicillium* associated to sunflower seed microbiota (Levitskaya et al., 2023; Selemani, 2025).
Detection of seed borne pathogens is an important step of integrated pest management strategies in sunflower crops (Guan et al., 2020). Thus, our research focused on detection and identification of the microbiota community associated with sunflower seeds (Dudoiu, 2016).

MATERIALS AND METHODS

The study was carried out on sunflower hybrids cultivated in Zimnicea, Teleorman County, with the aim of identifying pathogens present on seeds. The biological material was represented by seeds from sunflower hybrids EDS Belfis CLP, LG5077, QC Bravo, P64LE 99, P64LE 162, P64LE 280, Demetra and, Suria.
For pathogens isolation and identification, the seeds were incubated on PDA (Potato Dextrose Agar) culture medium at 24°C.
Four experimental variants were tested: untreated seeds, treated seeds with Lumisena 200 FS (200 g/l oxithiapiprolin), seeds washed with distilled water and seeds disinfected with ethanol (immersion for 2 minutes in 70% ethanol, rinsed with distilled water). Seeds treated with water and ethanol have been dried. Each variant was conducted on three replicates (10 seeds/plate/replicate) and the test was repeated twice.

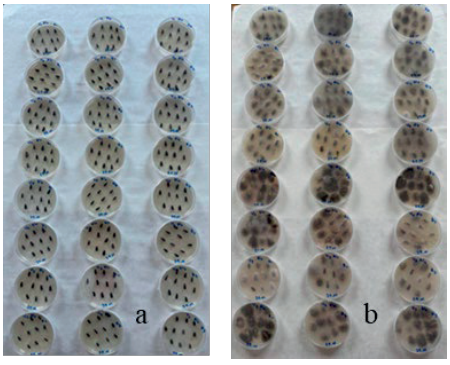

Figure 1. Setting up the experiment: a) day 1; b) day 7

The incidence of isolated colonies was determined by observations made at 3 and 7 days. Macroscopic observations of colonies were followed by light microscopy (Zeiss Primo Star microscope). Results are presented as fungal colonies frequency or incidence (%).

RESULTS AND DISCUSSIONS

The data presented in Table 1 shows the microbiota detected on sunflower tested seeds, the identified colonies belonging to the genera *Alternaria, Penicillium, Rhizopus* and *Aspergillus.*
Our study showed that in Lumisena treated seeds variants (LT) isolates of *Alternaria* spp. were present only in two hybrids, EDS Belfis CLP and P64LE99, and *Rhizopus* spp. occurred in LG5077. *Aspergillus* spp. and *Penicilium* spp. have not been detected (Table 1).
In untreated seeds variants (NT) isolates belonging to genera *Alternaria, Aspergillus* and *Rhizophus* were present in all tested hybrids. Colonies of *Penicilium* spp. have been not detected.
In the ethanol-treated seeds variants (ET), *Alternaria* spp. and *Aspergillus* spp. isolates were present in all hybrids. *Rhizopus* colonies were not present in Suria and QC Bravo and *Penicilium* colonies were present in QC Bravo and P64LE99 hybrids.
In distilled water treated seeds variants (WT), isolates of *Alternaria* spp. as well as those of *Aspergillus* spp. occurred in all hybrids. Colonies of *Aspergillus* were not detected on seeds of P64LE280 hybrid but in this variant *Penicilium* spp. isolates have been recorded. *Rhizophus* spp. colonies occurred in all hybrids, with one exception, Demetra.
The morphology of some colonies is presented in Figure 2.
Microscopic identification of isolates is shown in Figure 3.
The incidence of pathogens is shown in Table 2.
As can be seen, in the Lumisena 200 FS treated seeds isolates of *Alternaria* spp. were present in EDS Belfis CLS (20% incidence) and P64LE99 (3.3% incidence). No colonies of *Aspergillus* spp. have been detected. Isolates of *Penicillium* spp. had an incidence of 3.3% in the hybrid EDS Belfis CLS.
In the untreated seeds variants isolates of *Alternaria* spp. had the highest incidence in

Demetra hybrid (50%) and the lowest incidence in LG5077 hybrid (16.6%).
Isolates of *Aspergillus* spp. recorded the highest incidence in QC Bravo hybrid (33.3%), followed by EDS Belfis CLP and P64LE99 (3.33%). No colonies belonging to *Aspergillus* species were recorded in Demetra, LG5077 and P64LE162 hybrids.

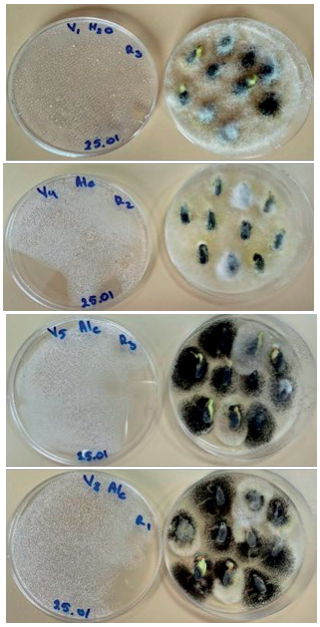

Figure 2. Fungal colonies developed on sunflowers seeds (after 7 days of incubation)

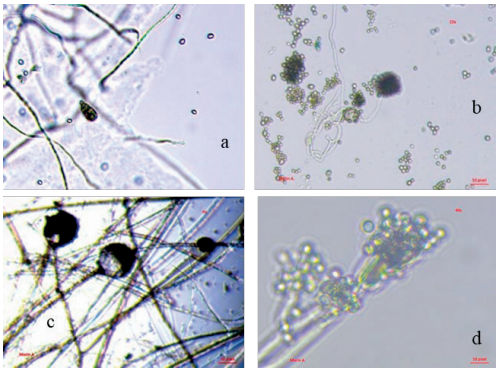

Figure 3. Microscopic identification of detected isolates: a. *Alternaria* spp. - conidia; b. *Aspergillus* spp. - conidia; c. *Rhizopus* spp. - sporangia; d. *Penicillium* spp. - conidiophore and conidia

In the ethanol-treated variants isolates of *Alternaria* spp. have been recorded with an incidence of 50% in Demetra and P64LE99 hybrids. A high incidence of *Aspergillus* spp. isolates has been recorded in EDS Belfis CLP (70%) and P64LE162 (73%) hybrids.
Low incidence values (3.3%) were recorded for *Penicillium* spp. isolates in P64LE99 and QC Bravo and P64LE280 hybrids.
In the water treated seeds variants have been recorded a high incidence of *Alternaria* spp. isolates (50% in LG5077) and *Aspergillus* spp. isolates (83% in QC Bravo). Colonies of *Penicillium* spp. were identified only in P64LE280 hybrid with 3.3% incidence.
The microbiota of the sunflower seeds have been represented by isolates belonging to genera of *Alternaria, Penicillium, Rhizopus* and *Aspergillus.* Our results are consistent with those reported in other studies. Thus, on sunflower seeds, different fungal species belonging to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Penicillium* and *Phomopsis* are reported (Mukhtar, 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*, *Phomopsis macdonaldii* (Abdullah & Al-Mosawi, 2010).
The major seed borne pathogenic fungi recorded in sunflower were *Alternaria alternata, A. helianthi*, *Rhizoctonia bataticola*, *Macrophomina phaseolina* and *Fusarium oxysporum*. Also, saprophytic fungi as *Aspegillus niger, A. flavus,* and *R. stolonifer* were reported (Patil et al., 2018).
The presence of fungal species as *Alternaria alternata, A. helianthi, Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Drechslera tetramera*, *Fusarium solani, F. moniliforme*, *Macrophomina phaseolina*, *Mucor mucedo, Penicillium* spp. and *Rhizopus* spp. on seeds from different sunflower cultivars was found to reduce seed germination by 10-20% and seedling mortality by 10-12% (Afzal et al., 2010).

Table 1. Detected microbiota in sunflower seeds

Var.	Isolates															
	Alternaria spp.				*Aspergillus* spp.				*Rhizopus* spp.				*Penicillium* spp.			
	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT
V1	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-
V2	-	+	+	+	-	+	+	+	+	+	-	+	-	-	-	-
V3	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-
V4	-	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-
V5	-	+	+	+	-	+	+	+	+	+	-	+	-	-	+	-
V6	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-
V7	-	+	+	+	-	+	+	-	+	+	+	+	-	-	-	+
V8	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-

V1 - Hybrid EDS Belfis CLP; V2 - Hybrid Suria; V3 - Hybrid Demetra; V4 - LG5077; V5 - Hybrid QC Bravo; V6 - Hybrid P64LE99; V7 - Hybrid P64LE280; V8 - Hybrid P64LE162; LT - Lumisena 200 FS treatment; NT - untreated seeds; ET - ethanol treatment; WT - Water treatment.

Table 2. Incidence of fungal colonies on sunflower seeds after 7 days of incubation

Var.	Isolates incidence (%)															
	Alternaria spp.				*Aspergillus* spp.				*Rhizopus* spp.				*Penicillium* spp.			
	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT
V1	20	30	20	23	-	3.3	70	57	TC	TC	TC	TC	3.3	-	-	-
V2	-	30	40	17	-	6.6	60	70	PC	TC	-	TC	-	-	-	-
V3	-	50	50	17	-	-	50	73	PC	TC	PC	-	-	-	-	-
V4	-	16.6	30	50	-	-	50	23	-	TC	TC	TC	-	-	-	-
V5	-	30	33.3	17	-	33.3	53	83	TC	TC	-	TC	-	-	3.3	-
V6	3.3	23.3	50	37	-	23.3	33.3	50	TC	TC	TC	TC	-	-	3.3	-
V7	-	20	47	23	-	3.3	17	-	TC	TC	PC	TC	-	-	-	3.3
V8	-	20	20	36	-	-	73	60	PC	TC	PC	PC	-	-	-	-

V1 - Hybrid EDS Belfis CLP; V2 - Hybrid Suria; V3- Hybrid Demetra; V4 - LG5077; V5 - Hybrid QC Bravo; V6 - Hybrid P64LE99; V7 - Hybrid P64LE280; V8 - Hybrid P64LE162; LT - Lumisena 200 FS treatment; NT - untreated seeds; ET - Ethanol treatment; WT - Water treatment; TC - total colonization in Petri plates; PC - partial colonization in Petri plates.

CONCLUSIONS

The microbiota of the sunflower seeds have been represented by isolates belonging to genera of *Alternaria, Penicillium, Rhizopus* and *Aspergillus*.

The biological material was represented by seeds from sunflower hybrids EDS Belfis CLP, LG5077, QC Bravo, P64LE 99, P64LE 162, P64LE 280, Demetra and, Suria.

The highest fungal diversity was recorded on untreated seeds. Thus, on these variants, the highest incidence was recorded for *Alternaria* spp. isolates (50%) on Demetra seeds followed by *Aspergillus* spp. isolates (33.3%) on QC Bravo seeds.

Lumisena 200 FS treatment reduced seeds fungal contamination with *Alternaria* spp. isolates compared to untreated seeds. Thus, in EDS Belfis CLP hybrid the incidence of *Alternaria* colonies was reduced from 30% at 20% and in in P64LE99 hybrid from 23.3% to 3.3%. No isolates of *Alternaria* spp. have been detected in the other tested variants.

In ethanol-treated seeds isolates of *Alternaria* spp. and *Aspergillus* spp. have been recorded, with high incidence values in all variants (20-50% for *Alternaria* isolates and 17-73% for *Aspergillus* isolates).

In water-treated seeds variants, high incidence of *Alternaria* spp. isolates (20-50%) and *Aspergillus* spp. isolates (23-83%) have been recorded.

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