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Effect of Some Micromycetes on Seed Germination and Seedling Growth in Sunflower

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ABSTRACT

Seed-borne fungi are reported to cause seed rot, damping off, and other diseases at germination, leading to reduced seed viability and impaired seedling establishment. On the other hand, many endophyte seed-borne fungi have mutualistic symbiotic associations with plants by inducing tolerance to biotic and abiotic stresses. In this research, the influence of some fungi (*Alternaria alternata, Aspergillus niger, Fusarium oxysporum* and *Rhizopus arrhizus*) on germination and early seedling development of sunflower under the artificially induced hydric stress was studied. Results revealed genotype-specific responses. *Alternaria alternata* significantly inhibited seed germination (up to -40%) and shoot growth across all genotypes, confirming its high virulence. In contrast, *R. arrhizus* and *F. oxysporum* showed moderate or stimulatory effects, especially on root elongation and biomass accumulation. Under hydric stress, some fungi (particularly *R. arrhizus*) increased shoot and root length, indicating a possible role in water stress tolerance.

Further exploration of these fungal interactions is essential for elucidating their potential applications in agriculture, particularly in strategies aimed at enhancing crop resilience and productivity.

Keywords: sunflower, seedling, biomass, fungal strain, SOD activity.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a key commercial oilseed crop worldwide. However, its yield is significantly limited by both biotic and abiotic factors, particularly in the context of actual climate change. The crop is affected by more than 30 pathogens - fungi, bacteria, and viruses. Among these, fungal stains, including seed-borne micromycetes, are the most detrimental, causing substantial reductions in seed yield and oil quality. Major fungal pathogens reported on sunflower seeds include *Alternaria helianthi*, *Rhizoctonia bataticola, Macrophomina phaseolina*, *Plasmopara halstedii*, *Fusarium oxysporum* and *Alternaria alternata*, alongside saprophytic fungi like *Aspergillus flavus*, *A. niger*, *Rhizopus arrhizus*, *Rhizopus stolonifera*, *Penicillium*, and *Curvularia*. Seed-borne fungi affect seed germination, root and shoot growth, seedling vigor, plant population density, as well as contribute to seed decay and the production of mycotoxins (Ghoneem et al., 2014; Chandel and Kumar, 2017; Patil et al., 2017).

They can significantly reduce the nutritional quality of sunflower seeds by decreasing protein, carbohydrate, and cholesterol content, as well as iodine values, while increasing acid levels (Sharfun-Nahar et al., 2005). Thus, species like *Rhizopus*, including *R. stolonifer* and *R. oryzae*, cause head rot in sunflower plants, which results in severe oil quality issues. Sunflower seeds from plants infected with *Rhizopus* head rot exhibited a free fatty acid content of 19.4%, compared to just 0.8% in oil from healthy seeds. Additionally, the oil from infected seeds contained higher levels of palmitic, stearic, arachidic, behenic, and lignoceric fatty acids (Thompson et al., 1980).

Alternaria spp., such as *A. helianthi*, *A. alternata*, *A. leucanthemi*, *A. zinnia* and *A. tenuissima* have been reported as pathogens causing *Alternaria* leaf blight of sunflower (Raranciuc and Joiţa-Păcureanu, 2002; Zhang et al., 2021). Numerous *Fusarium* species (*F. oxysporum*, *F. solani*, *F. moniliforme* and *F. culmorum*) are responsible for sunflower wilt, which affects roots, stems, and leaves (Yang et al., 2024).

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On the other hand, different fungi, such as *Aspergillus, Penicillium, Fusarium* species, can also live within the plant tissues (e.g., in the roots, stems, or leaves) without causing any harm or symptoms in the plant. Farhat et al. (2023) identified several such endophytic fungi from healthy sunflower plants, including *Aspergillus terreus*, *Curvularia lunata*, *C. hawaiiensis*, *Macrophomina phaseolina*, *Fusarium solani*, *Talaromyces assiutensis*, and *T. trachyspermus* (Farhat et al., 2023).

Different studies revealed beneficial role of endophytic fungi, highlighting their potential for plant health and growth. Thus, fungal endophytes enhance plant resilience to abiotic stresses, improve nutrient uptake, and contribute to plant growth, including increased seed germination and early seedling growth (Li et al., 2019). Various strains of *Aspergillus*, including *A. niger* and *A. flavus*, have demonstrated the ability to enhance plant growth through diverse mechanisms (Maia et al., 2024). The beneficial effects of these fungi are primarily attributed to the bioactive compounds they produce, which stimulate plant growth and contribute to adaptive responses or immunity (Lugtenberg et al., 2016). Among the various metabolites produced by endophytic fungi, gibberellins (GA) and indole-3-acetic acid (IAA) are key compounds that modulate defense hormones such as salicylic acid (SA) and jasmonic acid (JA), thereby enhancing plant resistance to pathogens, insect attacks, and abiotic stresses like drought, salinity, and heat. Additionally, enzymes produced by fungi, such as amylase, cellulase, and protease contribute significantly to ecosystem functioning by aiding in the degradation of organic matter and the release of essential nutrients (Waqas et al., 2015; Evstatieva et al., 2020; Ravichandran et al., 2022).

Endophytic fungi *Penicillium citrinum* and *Aspergillus terreus* 5 enhanced sunflower growth and significantly reduced stem rot severity caused by *Sclerotium rolfsii* by modulating hormone signaling and defense responses (Waqas et al., 2015). Endophytic fungus *Rhizopus oryzae* demonstrated significant potential to enhance thermal stress tolerance and promote growth in sunflower and soybean (Ismail et al., 2020a).

In this context, the influence of several fungal strains (*Alternaria alternata*, *Rhizopus arrhizus*, *Fusarium oxysporum*, and *Aspergillus niger*), applied individually and in combination, on sunflower seed germination and early seedling growth parameters was analyzed under both absence and presence of hydric stress artificially induced by PEG 6000.

MATERIAL AND METHODS

Plant Material and Experimental Design

Three local sunflower hybrids, conventionally noted H2, H3 and H4, with distinctive reaction to abiotic and biotic factors in the field, were evaluated under controlled laboratory conditions for their response to *Alternaria alternata*, *Rhizopus arrhizus*, *Fusarium oxysporum*, *Aspergillus niger*, and a fungal mix, both in the presence and absence of hydric stress.

Sunflower seeds were surface sterilized twice with 70% ethanol for 30-60 seconds, then rinsed 3-5 times with distilled water. Afterward, a 20% sodium hypochlorite solution was applied for 5 minutes (or 10% sodium hypochlorite for 10 minutes) for further disinfection, followed by another 3-5 rinses with distilled water to remove residual NaClO. The substrate, consisting of perlite and sand mixed in a 1:1 ratio (25:25 ml), was autoclaved three times to ensure effective sterilization. Surface-disinfected sunflower seeds were sown in pots at a density of 30 seeds per pot, with two replicates assigned to control conditions (no water stress) and two for hydric stress treatments.

A fungal spore suspension (10^6 spores/mL), prepared by washing 14-day-old cultures grown on oblique malt-agar columns with sterile distilled water, was inoculated into the pots after sowing. The micromycetes used in this study were isolated from infected plant tissue and soil, and were provided by colleagues from the Institute of Microbiology and Biotechnology, Technical University of Moldova. Additionally, two non-inoculated control variants (one subjected to hydric

stress and one maintained under non-stress conditions) in two replicates were used. The pots were covered with sterile filter paper moistened with sterile distilled water, placed in polyethylene bags, and incubated at 25°C in darkness in a growth chamber for three days. After incubation, the germinated seeds were counted. Then, 100 mL of sterile sand was added to each pot, and the contents were irrigated twice a week with 50 mL of half-strength Hoagland nutritive solution. The cultivation conditions were set to a temperature regime of 25°C, with a photoperiod of 14/10 hours (light/dark).

At the stage of the first pair of true leaves, half of the sunflower plants, both fungal-inoculated and non-inoculated (two pots per variant), were subjected to water stress. Plants in the non-stress variants were watered with 80 mL of distilled water, while those in the stress variants received 80 mL of 20% polyethylene glycol (PEG-6000) solution.

Assessment of Seedling Growth and Stress Tolerance Indices in Sunflower

After three days, sunflower seedlings were harvested and morphometric parameters, including root and shoot lengths, as well as fresh and dry biomass, were recorded. Dry weights were determined after drying the seedlings at 70°C at a constant weight. From each sample in every replication, five normal seedlings were randomly selected for analysis.

Germination rate (GR) was calculated as follows: GR (%) = (Number of germinated seeds / Total seeds) × 100. Based on these measurements the plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), fresh (FMSI) and dry matter stress tolerance index (DMSI) were calculated according to Ashraf et al. (2006), (x), as following:

– Plant Height Stress Index (PHSI) = (Height of stressed plants / Height of control plants) × 100;

– Root Length Stress Index (RLSI) = (Root length of stressed plants / Root length of control plants) × 100;

– Fresh Matter Stress Index (FMSI) = (Fresh weight of stressed plants / Fresh weight of control plants) × 100;

– Dry Matter Stress Index (DMSI) = (Dry weight of stressed plants / Dry weight of control plants) × 100.

Determination of Superoxide Dismutase (SOD) Activity

Leaf samples (0.5 g) were homogenized in 2 ml of 10 mM Tris-HCl buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was centrifuged at 12,000 rpm for 10 minutes at 4°C, and the resulting supernatant was used as the enzyme source for SOD activity determination.

SOD activity was assayed by measuring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT), according to the method of Beyer and Fridovich (1987). The reaction mixture (3 ml) contained 10 mM Tris-HCl buffer (pH 7.8), 130 mM methionine, 2 mM NBT, 0.6 mM riboflavin, 0.1 mM EDTA, and 100 µl of the enzyme extract. The reaction was initiated by illuminating the mixture for 15 minutes under a consistent light source and then stopped by transferring the samples to darkness for 10 minutes. Absorbance was measured at 560 nm against a control lacking the enzyme extract. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction under the assay conditions.

Difference significance of mean values was analyzed by Student's t-test ($p<0.05$). Data was graphed as mean ± SDs.

RESULTS AND DISCUSSION

Effect of fungal inoculation on sunflower seed germination rate. Fungal strains significantly affect sunflower cultivation and seed production, particularly during the early germination stages, which lead to low seedling emergence and subsequent field diseases, which result in substantial economic losses. The analysis of sunflower seed germination in the presence of fungi reveals varying degrees of inhibition depending on the fungal species and genotypes, the most affected being the hybrid H4 (Figure 1).

Alternaria alternata and the fungal mix exhibited a significant negative impact on all

genotypes. The most pronounced reduction in germination (up to 40% comparative to control) was observed in the case of *A. alternata*, which is consistent with previous reports. Thus, *Alternaria* spp. have been shown to decrease both germination and seedling development in rapeseed (*Brassica napus*) (Soomro et al., 2020), while the metabolites synthetized by *A. alternata* significantly reduced the germination of sesame seeds (Bibi et al., 2023). It was, also, reported that the reduced germination rate in sunflower seeds was closely linked to the higher number of seeds infected with *Alternaria* spp. (Zală et al., 2010).

Aspergillus niger showed significant inhibitory effect (around 45%) only in the case of genotype H4. Interestingly, *Fusarium oxysporum* and *Rhizopus arrhizus* exhibited relatively moderate or limited effects on germination, which contrasts with the findings of Jadhav et al. (2023), who noted a 30% decrease in germination of infected lentil seeds compared to healthy ones (Jadhav et al., 2023) and the results of Gawarkar et al. (2023), who reported that *F. oxysporum* significantly reduced germination (about 30% compared to uninoculated seeds), shoot length (44-47%), and root length (67%) in sesame. Furthermore, *Rhizopus arrhizus* is known to produce various secondary metabolites, including indole-3-acetic acid and gibberellic acid, which play a significant role in plant germination and growth regulation (Evstatieva et al., 2020).

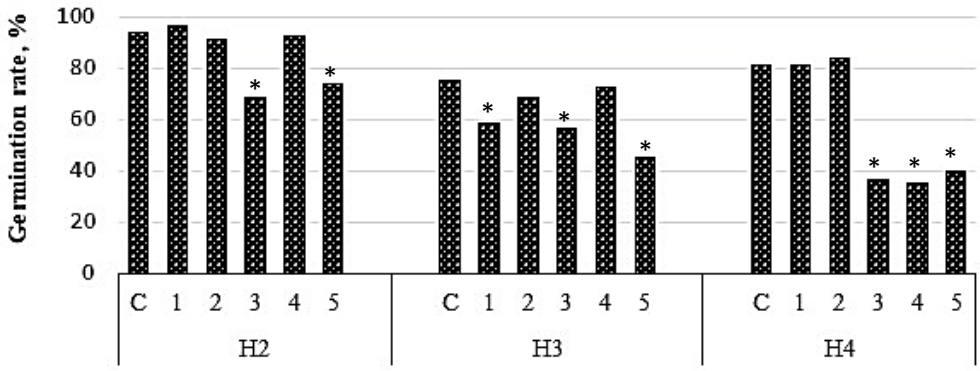

Figure 1. Germination rate of sunflower genotypes after inoculation with fungal strains (*1 - Fusarium oxysporum, 2 - Rhizopus arrhizus, 3 - Alternaria alternata, 4 - Aspergillus niger, 5 - Fungal mix).* Asterisk (*) indicates statistically significant change over control at p≤0.05 (t-test)

Exposure to mixed fungal inoculum resulted in the severe germination suppression (by 20-41% to control) of all genotypes, suggesting additive or synergistic interactions among pathogens. These results underline the importance of pathogen-specific and combined infection studies when assessing seed health and support the development of targeted antifungal strategies in sunflower cultivation.

Morphological traits of sunflower seedlings co-cultivated with fungal strains under non-hydric stress conditions. The effects of fungal pathogens on sunflower seedlings morphological parameters, such as shoot and root length, and biomass accumulation was analyzed.

Fungal pathogens significantly reduced shoot length in sunflower across all hybrids, with the severity depending on both the fungal species and genotype (Table 1). The height of the plants ranged from 8.5-11.1 cm, 7.7-14.6 cm, and 8.1-12.0 cm for H4, H2 and H3. *Alternaria alternata* showed the highest inhibitory effect, with reductions ranging from 16.6% in H4 to 40.3% in H2 (Figure 2), confirming its high virulence.

A. **B.**

Figure 2. The aspect of **s**unflower genotype H2 grown in the presence of fungal strains under the absence (A) and presence (B) of hydric stress: *c - control, 1 - F. oxysporum, 2 - Rh. arrhizus, 3 - A. alternata, 4 - A. niger, 5 - Fungal mix*

Both *Fusarium oxysporum* and *Rhizopus arrhizus* caused moderate decreases in sunflower seedling growth (approximately 20-25%), while *Aspergillus niger* exhibited a dual effect: an inhibitory effect in the *H4* genotype (-23.6%) and a stimulatory effect in the other two hybrids (Figure 3). Similarly, a significant increase in most of the growth parameters were found in sunflower and soybean seedlings inoculated with *A. niger* as compared to *A. niger* - free under heat stress (Ismail et al., 2020b). The fungal mixture resulted in minor declines across the genotypes, with *H3* being the most affected (21.2%). Shoot length exhibited low variability (CV 1.1-3.1%), indicating consistent growth responses.

Root length exhibited a more variable pattern under fungal stress (Table 1). In the hybrid H4, root elongation increased across all treatments, most notably with *Rhizopus arrhizus* (+26.6%) and *Fusarium oxysporum* (+13.5%), suggesting potential root stimulation. Similarly, an increase in root elongation was reported in barley and sweet clover (Brazhnikova et al., 2021). In contrast, H2 showed negative responses, with the strongest reductions caused by the fungal mix (-17.3%). H3 displayed mixed responses, including an increase under *Alternaria* (+12.6%) and suppression from *Fusarium* (-20.5%). Overall, root length appeared more resilient than shoot length, supported by low variability (CV 2.7-4.8%).

Table 1. Morphometric parameters of sunflower genotypes grown in the presence of fungal strains under non-hydric stress conditions

Sunflower hybrid	Control	*F. oxysporum*	*Rh. arrhizus*	*A. alternata*	*Asp. niger*	*Fungal mix*
Shoot length, cm						
H2	12.9±0.4	12.0±0.1*	9.7±0.2*	7.7±0.2*	14.6±0.2*	12.5±0.2*
H3	10.3±0.2	8.3±0.2*	9.2±0.2*	9.6±0.2*	12.0±0.3*	8.1±0.2*
H4	11.1±0.2	9.0±0.1*	8.5±0.2*	9.3±0.2*	8.5±0.2*	10.6±0.1*
Root length, cm						
H2	6.0±0.2	5.5±0.1*	5.6±0.2*	5.3±0.2*	5.7±0.2*	5.0±0.2*
H3	6.3±0.2	5.0±0.2*	5.2±0.2*	7.1±0.3*	6.7±0.2*	5.4±0.3*
H4	4.7±0.2	5.4±0.1*	6.0±0.2*	5.0±0.2*	5.2±0.2*	5.1±0.2*
Fresh biomass, g						
H2	1.14±0.17	0.94±0.15^{ns}	1.09±0.13^{ns}	1.16±0.11^{ns}	1.05±0.16^{ns}	1.03±0.14^{ns}
H3	0.83±0.06	1.07±0.10*	0.86±0.11^{ns}	0.72±0.08^{ns}	0.90±0.02^{ns}	0.82±0.08^{ns}
H4	0.86±0.08	0.97±0.04*	1.04±0.07*	0.77±0.03*	1.01±0.07*	0.94±0.02^{ns}
Dry biomass, g						
H2	0.053±0.010	0.064±0.011^{ns}	0.066±0.010*	0.065±0.012^{ns}	0.055±0.008^{ns}	0.055±0.006^{ns}
H3	0.051±0.007	0.073±0.013*	0.056±0.010^{ns}	0.053±0.006^{ns}	0.055±0.007^{ns}	0.071±0.010*
H4	0.061±0.005	0.073±0.006*	0.082±0.002*	0.048±0.002*	0.069±0.004*	0.065±0.003^{ns}

An asterisk (*) indicates a statistically significant difference from the control at $p \leq 0.05$, as determined by a t-test; (^{ns}) - indicates no significant differences.

These results highlight the differential impact of fungal pathogens on early sunflower development and emphasize the importance of genotype-specific responses in disease management strategies.

Fresh and dry biomass weights varied across treatments and genotypes, with statistically significant increases observed particularly in the hybrid *H4*. Inoculation with *Fusarium oxysporum* and *Rhizopus arrhizus* led to notable increases in fresh (ranging from 12.8% to 29.2%) and dry biomass (ranging from 19.7% to 41.2%), with *Rhizopus* contributing increases of 24% to 34.4% depending on the genotype. *Aspergillus niger* showed a moderate positive effect, while *Alternaria alternata* had a negative influence on biomass accumulation.

It is well known that reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, accumulate in higher quantities under stress conditions, leading to oxidative damage of proteins, lipids, nucleic acids, and other cellular components. To counteract this, plants activate an enzymatic antioxidant defense system comprising key enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbic acid oxidase (AAO), which function to detoxify excess ROS. Among these, SOD serves as the first line of defense by converting highly reactive superoxide radicals into hydrogen peroxide, which is subsequently neutralized by CAT and AAO (Rajput et al., 2021; Nawaz et al., 2023). Thus, the assessment of SOD activity provides a sensitive and early indicator of oxidative stress and reflects the plant's ability to initiate an effective defense response. In the present study, SOD activity was measured to compare the antioxidant capacity of different sunflower genotypes under fungal-induced stress, thereby elucidating their role in stress adaptation and tolerance mechanisms.

A notable increase in SOD activity was observed in leaves of sunflower plants inoculated with *Fusarium oxysporum* and *Rhizopus arrhizus*, with values 25.8% and 36.8% higher than the control, respectively, depending on the hybrid (Figure 3). These increases correlated with moderate increasing in biomass accumulation and root elongation, suggesting a well-regulated oxidative response that may support stress adaptation. In contrast, in the case of *Alternaria alternata* the differences in SOD activity are especially insignificant, indicating a less effective antioxidant defense and a higher level of stress-induced cellular damage, which led to a strong reduction in some grow parameters (shoots length, biomass accumulation). A similar antioxidant response was observed in *Aspergillus niger* inoculated sunflower and soybean seedlings, where significant increases in antioxidative enzymes activities were found at 40°C compared to endophyte-free controls (Ismail et al., 2020b).

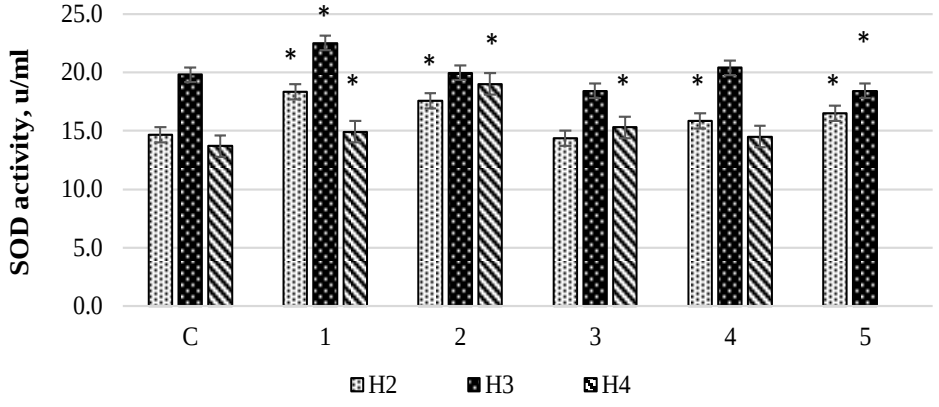

Figure 3. Activity of SOD in sunflower seedlings grown in the presence of fungal strains (*c - control, 1 - F. oxysporum, 2 - Rh. arrhizus, 3 - A. alternata, 4 - A. niger, 5 - Fungal mix).* An asterisk (*) indicates a statistically significant difference from the control at p 0.05 (t-test)

Morphological traits of sunflower seedlings co-cultivated with fungal strains under hydric stress conditions. It is well established that hydric stress significantly impacts sunflower development, affecting both growth and physiological traits, as well as seed composition. Previous studies have reported reductions in leaf area, root volume, chlorophyll content, and biomass, accompanied by increases in peroxidase activity and root/shoot ratio, along with alterations in fatty acid profiles (Petcu et al., 2001). In the present study, shoot length was generally more variable under hydric stress, with some fungi demonstrating reduced negative influence or even exhibiting potential growth-promoting effects. Thus, *Rhizopus arrhizus* increased shoot length in H4 from 9.8 cm (control) to 10.5 cm (Table 2), this value being approximatively similar with those established in the control variant (without PEG) In contrast, *Alternaria* reduced it drastically to 6.9 cm (-29.6%).

In most cases, plants inoculated with fungal strains exhibited greater root length under water stress compared to non-inoculated control, with the highest increases revealed in the presence of *Fusarium oxysporum* (+29% in H2), *Rhizopus arrhizus* (+16% in H4), and the fungal mix. Increased root length is often regarded as a key adaptive response in plants subjected to water-limited conditions, and as such, this response is considered beneficial for survival under ecological drought stress. This trait enhances the plant's ability to access water from deeper soil layers, which is critical for maintaining hydration and improving drought tolerance (Voothuluru et al., 2024).

Enhancement of plant biomass is a key indicator of crop performance under stress conditions. Fungal inoculation affected biomass accumulation in sunflower seedlings under hydric stress in a genotype-dependent manner, although most changes in dry biomass were statistically insignificant. *Rhizopus arrhizus* demonstrated a biostimulant effect, the dry biomass weight being by up to 33% in H4 and 32% in H3 higher compared to control. Similarly, *Fusarium oxysporum* promoted dry biomass accumulation in H2, with a 35.9% increase. In contrast, *Alternaria alternata* and *Aspergillus niger* showed inhibitory effects on dry biomass, reducing it by 25-28%. These results suggest that certain fungal strains, particularly *R. arrhizus*, may enhance sunflower performance under drought, while others, like *A. alternata*, may intensify stress-induced growth limitations.

Table 2. Morphometric parameters of sunflower genotypes grown in the presence of fungal strains under hydric stress conditions

Sunflower hybrid	Control	*F. oxysporum*	*Rh. arrhizus*	*A. alternata*	*Asp. niger*	*Fungal mix*
Shoot length, cm						
H2	12.2±0.2	8.3±0.2*	10.2±0.1*	10.8±0.2*	12.5±0.2*	11.6±0.2*
H3	7.8±0.6	8.9±0.6*	8.4±0.6*	8.1±1.0*	8.4±0.7*	8.5±0.7*
H4	9.8±0.3	8.2±0.2*	10.5±0.3*	6.9±0.3*	8.5±0.2*	7.8±0.2*
Root length, cm						
H2	5.8±0.2	7.5±0.1*	4.7±0.2*	6.3±0.2*	6.7±0.2*	7.3±0.2*
H3	5.8±0.6	5.7±0.3*	5.9±0.6*	5.8±0.4*	6.6±0.4*	4.6±0.6*
H4	5.0±0.2	5.3±0.2*	5.8±0.2*	5.3±0.3*	4.7±0.2^{ns}	4.6±0.1*
Fresh biomass, g						
H2	1.02±0.08	0.79±0.09*	0.62±0.09*	0.87±0.14*	0.79±0.11*	0.89±0.14^{ns}
H3	0.76±0.09	0.75±0.10^{ns}	0.57±0.06*	0.60±0.08*	0.86±0.10^{ns}	0.86±0.11 ^{ns}
H4	0.76±0.08	0.79±0.07^{ns}	0.91±0.05*	0.89±0.05*	0.89±0.04*	0.66±0.02*
Dry biomass, g						
H2	0.064±0.011	0.087±0.010*	0.056±0.004^{ns}	0.059±0.006^{ns}	0.046±0.006*	0.060±0.006^{ns}
H3	0.053±0.005	0.061±0.008 ^{ns}	0.070±0.010*	0.045±0.003*	0.040±0.008*	0.057±0.006^{ns}
H4	0.060±0.006	0.057±0.002^{ns}	0.080±0.001*	0.062±0.003^{ns}	0.056±0.003^{ns}	0.056±0.005^{ns}

An asterisk (*) indicates a statistically significant difference from the control at $p \leq 0.05$, as determined by a t-test; (^{ns}) - indicates no significant differences.

In many cases, particularly in sunflower hybrid samples inoculated with *Fusarium oxysporum* and *Rhizopus arrhizus*, the PHSI, RLSI, FMSI, and DMSI under combined (hydric and biotic) stress conditions were higher or comparable to those recorded under biotic stress alone (Figure 4).

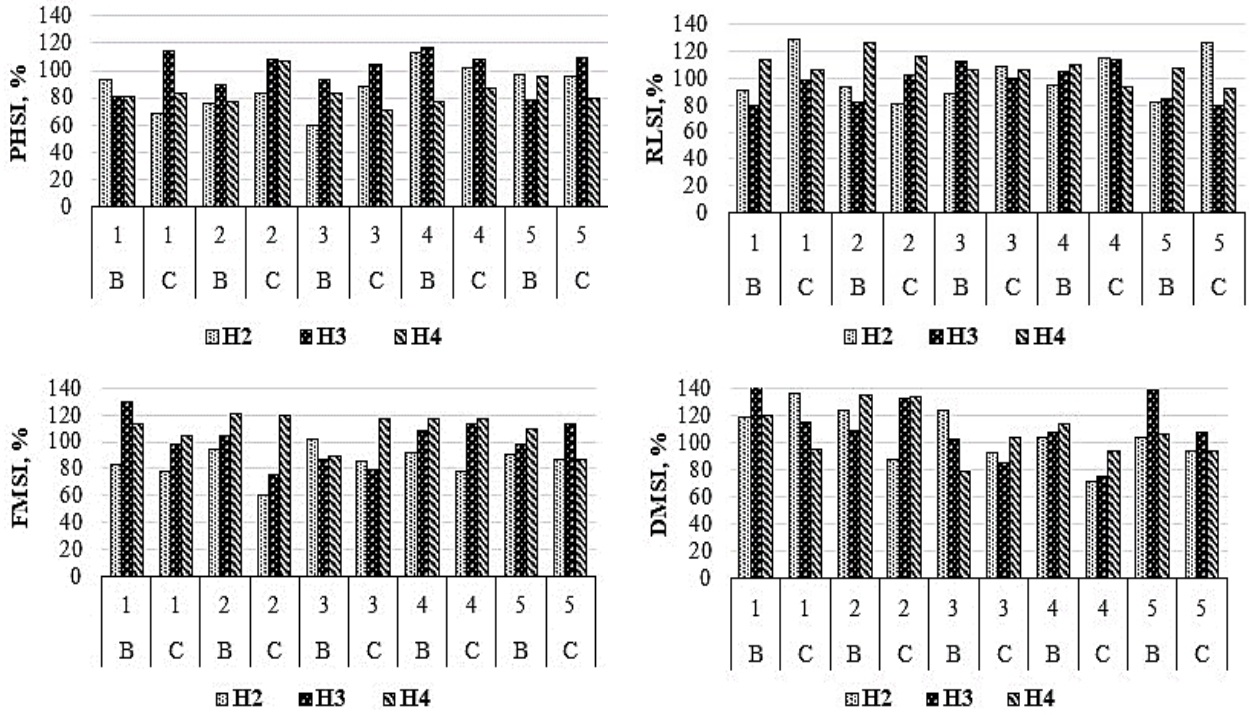

Figure 4. Effect of fungal strain on plant Height Stress Index (PHSI), Root Length Stress Index (RLSI), Fresh (FMSI) and Dry Matter Stress Index (DMSI) in sunflower hybrids under the absence (B - biotic stress) and presence (C - combined stress) of hydric stress: *1 - F. oxysporum, 2 - Rh. arrhizus, 3 - A. alternata, 4 - A. niger, 5 - Fungal mix.*

This suggests a potential mitigating effect of fungal inoculation on the impact of combined stress. It is known that species of the genus *Rhizopus* produce various secondary metabolites, including phytohormones such as indole-3-acetic acid, gibberellic acid, which stimulate seed germination, enhance the development of xylem and root tissues and promote stem elongation. The presence of phytohormones auxins and gibberellins was confirmed in the cell-free supernatant of *Rh. arrhizus* KB-2 and shown to positively influence seed germination and root development (Evstatieva et al., 2020).

Furthermore, the *Rh. arrhizus* strain used in this study is a known producer of hydrolytic enzymes such as cellulases and amylases, and has been patented for its high production of lipases and pectinases (Deseatnic et al., 2003) which may contribute to improved nutrient availability and plant resilience under stress conditions.

CONCLUSIONS

The inoculation of sunflower seeds with various fungal strains has a significant impact on germination and seedling growth, with varying effects depending on the fungal species and sunflower genotype. *Alternaria alternata* had a negative effect, reducing germination rates and seedling development. This agrees with earlier research showing its harmful impacts on other crops. *Aspergillus niger* has both an inhibiting and a promoting effect, depending on sunflower genotype. The fungal mixture led to severe germination suppression, highlighting the importance of considering combined fungal infections in sunflower cultivation. Furthermore, fungal inoculation under hydric stress showed that *Rhizopus arrhizus*, in particular, enhanced root length and biomass accumulation, suggesting a potential biostimulant effect. These results emphasize the complex interaction between fungal pathogens and

sunflower genotypes, suggesting that some fungi may help mitigate the effects of stress, while others exacerbate it.

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