MICROMYCETES ASSOCIATED WITH SUNFLOWER SEEDS DURING STORAGE PERIOD

Roxana DUDOIU[1], Carmen LUPU[1], Vasile JINGA[1], Stelica CRISTEA[2], Florentin CHITORAN[3]

[1]research Development Institute For Plant Protection, Bucharest, Romania
[2] University Of Agronomical Sciences And Veterinary Medicine, Faculty Of Agronomy, Bucharest, Romania
[3] S.C. Agrotehnic, Păulești, Prahova County, Romania

** roxyanna_21@yahoo.com*

ABSTRACT

Sunflower seeds can become contaminated with fungi which attack the plants at different stages of development and subsequently during harvesting and storage. An important role for the production of healthy crops is being played by quality seeds. Although the initial seed quality and storage environment are mandatory to prolong the shelf-life of seeds, the invasion of fungal pathogens causes various abnormalities like damaged seeds, undersized, rotted seeds and reduced in germinability. Fungal organisms play a significant role in infection, altering quality and longevity of seeds during the storage. The sunflower seeds losses recorded during storage period on worldwide scale according to FAO estimations are 10-15% of total production. In developing countries, due to reduced possibilities of implementing appropriate technologies, the reported damages during storage period may increase up to 30%. The paper work presents a study regarding the occurrence and development of specific storage micromycetes of sunflower achenes, collected from a deposit in Prahova County, Romania. Analysis performed on four sunflower hybrids' seeds, regarding the fungal load, revealed the occurrence of several pathogenic and saprophytic fungi belonging to the following genera: *Aspegillus, Fusarium, Alternaria*, *Stemphylium, Trichoderma*, *Cladosporium*, *Rhizopus*, *Mucor*, *Pencillium*, and *Sclerotinia*. All studied hybrids were contaminated with species of *Alternaria* and *Stemphylium,* the highest incidence noticed was for NK Adagio hybrid with 56 % of *Alternaria* spp. Placing on the market safe food and feed products is first and foremost a question of good management practices at each stage of the feed and food chain from primary production to final.

Key words: sunflower, micoflora, *Alternaria* spp., stored products

INTRODUCTION

Sunflower (*Helianthus annuus*) is one of the most cultivated oilseed crops worldwide. In 2015, Romania ranked first in the European Union both in terms of area and sunflower production, with a total harvest of 1.75 million tones obtained from one million hectares (www.paginadeagricultura.ro/). Compared to 2014, sunflower production decreased in 2015 by 19.7% due to the yield per hectare which fell by 19.6%.

Storage of sunflower seeds may last for various periods of time, when this product is kept for subsequent planting, oil processing or use in confectionery industry. A series of environmental factors, when improperly managed, could make sunflower seed storage questionable, particularly when long-term keeping is foreseen. Among the microbes that

interact with the seeds during their storage, fungi play a dominant role in decreasing quality and longevity of the seeds (Mardare et al., 2015, Cristea et al., 2004, Cristea et al., 2009, Mardare, 2014) Fungi cause various abnormalities like damaged seeds, undersized, rotted seeds and reduced in germinability. Fungal organisms play a significant role in infection, altering quality and longevity of seeds during the storage. In order to develop the list of storage fungi of sunflower, sunflower seeds were screened to study the incidence of fungi which gave the occurrence of *Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme, Penicillium citrinum, Macrophomina phaseolina* and *Rhizopus nigricans* with sunflower seeds (Afzal R., et al., 2010). Same types of fungi including species of *Cladosporium* and *Drechslera* have been reported from sunflower seeds. (Kakde R. B. et al., 2012). *Absidia corymbifera, Alternaria alternata, Aspergillus flavus, A. niger, A. terreus, Chaetomium bostrychodes, C. globosum, Emericella nidulans, Fusarium pallidoroseum, F. solani, Macrophomina phaseolina, Penicillium spp., Rhizoctonia solani* and *Rhizopus stolonifer* were predominantly isolated from sunflower (Nahar, S. et al., 2005).

Sunflower seed storage needs particular care, due to its high fat content, easily cracking shell, exposing the kernel to various alterations, such as germination loss, colonization of fungi and attacks by insects and mites. Seed moisture content is critical mainly if long-term storage is intended. Proper management of equilibrium between relative air humidity and seed moisture is an essential element, dictating need for seed drying at storage start and frequent aeration during storage (Beratlief, 1997). During the stockpile, a series of fungi appear on the seeds' surface, which generally not produce damages to the crop, whereas at the moment of tilling, they disappear due to the inauspicious soil conditions. However, certain situations have been encountered when the storage funguses have had a major influence while keeping the seeds in deposits. It can ultimately get to the germination diminution, so much that it can drop under the limit allowed by the law (specifically for seeding), and, in this case, the lot is entirely compromised and the loss is total. Also, the storage fungi negatively influenced seeds in process of field germination, leading to weakling plants, more susceptible to diseases (Zala et al., 2010).

MATERIALS AND METHODS

The research aimed to identify the spectrum of pathogens present on sunflower achenes, in order to determine the yield's health status after being harvest and recommend an effective treatment.

The biological material consisted in samples of sunflower seeds, naturally infected with pathogens and collected from a storage unit Prahova County, in South-Eastern part of Romania. The achenes belonged to four sunflower cultivars, two Romanian hybrids (Performer and Favorit) and two international ones (NK Adagio and NK Meldimi), adapted to growing conditions in this part of the country.

In order to study the micoflora, sunflower contaminating fungi were isolated using Ulster method (Hulea A., 1969) and identified with optical microscope, after 12 days of growth. Using the Ulster method there can be identified the majority of seed pathogens, regardless the species and type of seed. In separate Petri dishes of 10 mm diameter, were placed 15 wheat seeds on growth solid media (water-agar, 20 g/l, autoclaved 20' at 121 ºC), with space between the achenes in order to allow the development of fungi or bacteria. The water agar media was preferred due to its low nutrients composition which allows the fungi growth, but not its abundant sporulation. This is an important step in order to be able to isolate each fungus from the Petri dish multitude of pathogens. The dishes were kept at room temperature (22-24 ºC) and 12h/12h light conditions. After 7 days there were performed

macroscopic observations regarding the mycelia growth in Petri dishes, followed by optical microscope determinations, using a Zeiss Primo Star microscope. For further isolation and purification of each fungus was used the Potato-Dextrose-Agar medium (Hulea A., 1969). The determination of sunflower seeds' germination was performed on filter paper.

Figure 1. Sunflower seeds inoculated on water-agar medium, incubated for 7 days

RESULTS AND DISCUSSIONS

Microscopic examinations revealed a spectrum of fungi belonging to *Ascomycetes* and *Deuteromycetes* classes.(Gheorghies et al, 2001, Kieffer et al., 2000, Varga et al., 2009).

The data in table 1 presents the pathogen species detected on sunflower achenes belonging to *Alternaria* spp., *Penicillium* spp., *Stemphylium* spp., *Rhizopus* spp., *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Sclerotinia* spp., *Trichoderma* spp., and *Cladosporium* spp.

Table 1. Micoflora detected on sunflower achenes

The pathogenic agent	***Hybrid***			
	PERFORMER	FAVORIT	NK ADAGIO	NK MELDIMI
Alternaria spp.	+	+	+	+
Stemphylium spp.	+	+	+	+
Penicillium spp.	+	+	-	+
Rhizopus spp.	+	+	+	+
Aspergillus spp.	+	+	+	-
Fusarium spp.	-	-	-	+
Sclerotinia sclerotiorum	-	+	-	-
Mucor spp.	+	+	+	+
Trichoderma spp.	-	+	-	-
Cladosporium spp.	+	+	+	+

From our studies, it was found that pathogens *Alternaria* spp., *Stemphylium* spp., *Rhizopus* spp., *Mucor* spp. and *Cladosporium* spp. have populated the achenes of all studied sunflower hybrids. The fungi belonging to *Penicillium* were present on the following achenes hybrids: Performer, Favorit, NK Meldimi. Species of *Fusarium* were isolated from NK Meldimi hybrid achenes. *Aspergillus* spp. has populated seeds from Performer, Favorit and NK Adagio cultivars. Species of *Trichoderma* and *Sclerotinia* were observed on achenes from Favorit hybrid.

In figures 2 - 7 are presented microscopical observations captured with the Zeiss Primo Star microscope.

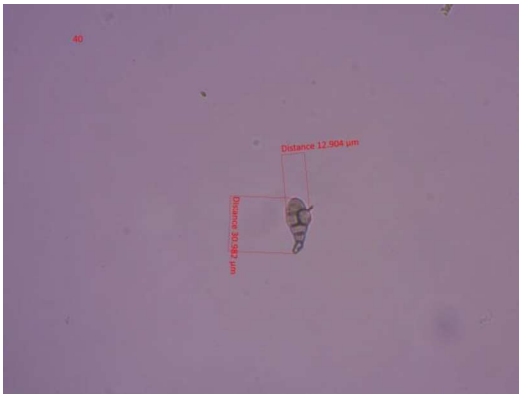

Figure 2. *Alternaria* sp. conidia

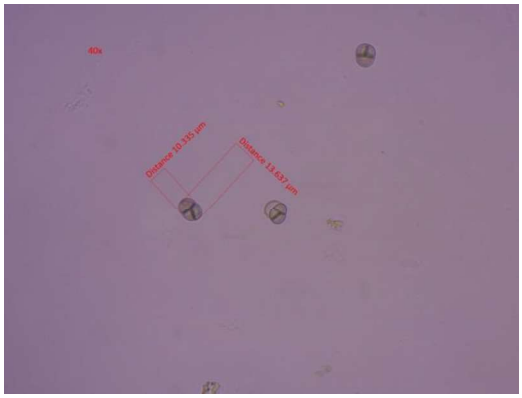

Figure 3. *Stemphylium* sp. conidia

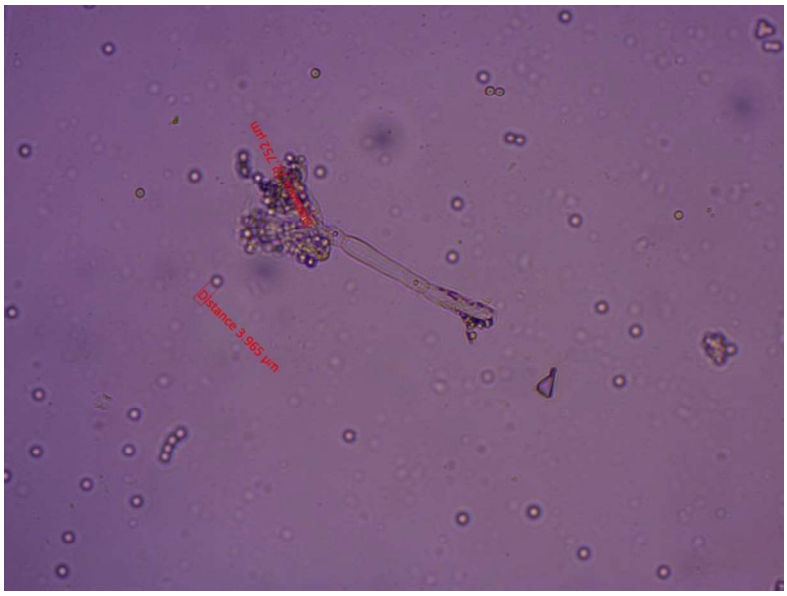

Figure 4. *Penicillium* sp. conidiophore and conidia

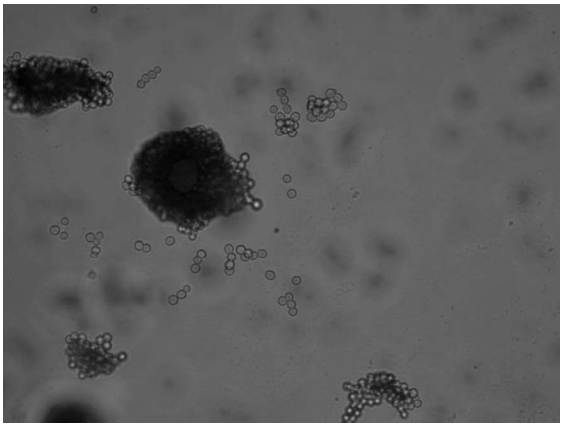

Figure 5. *Aspergillus* sp. conidias

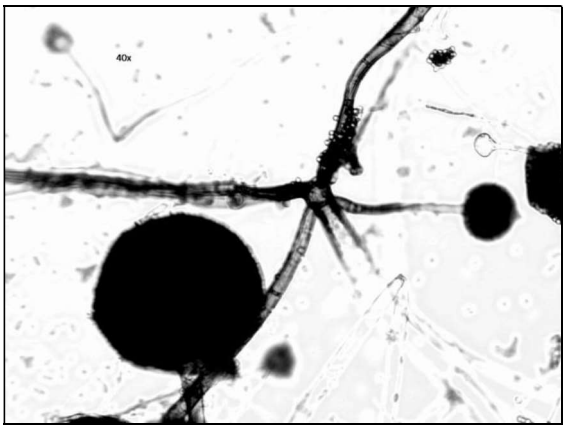

Figure 6. *Rhizopus* sp. sporangium

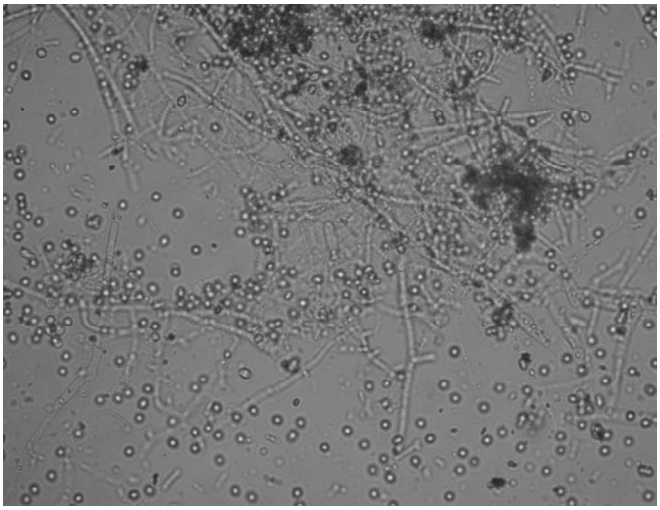

Figure 7. *Trichoderma* sp. fructifications

The incidence' rate of detected micoflora is presented in table 2. *Alternaria* spp. were present on sunflower achenes from all four hybrids, with highest values of frequency from all identified pathogens, respectively 56% for NK Adagio, 47% for NK Meldimi, 35% for Performer and 31% for Favorit hybrid.

The pathogens from *Stemphylium* spp. presented the highest values of incidence for Performer hybrid with 33% and Favorit hybrid with 30%. The other two hybrids presented 19% incidence for NK Adagio and 18% incidence for NK Meldimi.

Penicillium spp. were present in highest rate on sunflower achenes from NK Meldimi hybrid in 14%, 10% for Favorit hybrid and only 7% for Performer hybrid.

Species from *Mucor* and *Rhizopus* were detected on sunflower achenes belonging to all four hybrids, with relatively close incidence values. *Mucor* spp. was present in 3% on Performer and Favorit hybrids achenes, in 5% NK Adagio achenes and 6% the highest value for Favorit hybrid. *Rhizopus* spp. had the highest incidence value for Favorit hybrid, followed by Perfomer hybrid with 8% incidence, NK Adagio with 7% incidence and NK Meldimi with 4% incidence.

Fusarium sp. was detected with a low incidence of 5% only for NK Meldimi hybrid; Also *Trichoderma* sp. and *Sclerotina sclerotiorum* were present in 3% respectively 2% on sunflower achenes from Favorit hybrid.

Cladosporium spp. were detected with low incidence values on all sunflower hybrids achenes.

Table. 2 The micoflora's incidence detected on sunflower achenes

Fungi *(observations after 9 days)*	***Pathogens' incidence on hybrid (%)***			
	PERFORMER	FAVORIT	NK ADAGIO	NK MELDIMI
Alternaria spp.	35	31	56	47
Stemphylium spp.	33	30	19	18
Penicillium spp.	7	10	0	14
Rhizopus spp.	8	10	7	4
Aspergillus spp.	3	2	3	0
Fusarium spp.	0	0	0	5
Sclerotinia sclerotiorum	0	2	0	0
Mucor spp.	3	6	5	3
Trichoderma spp.	0	3	0	0
Cladosporium spp.	4	3	3	4
Other pathogens	7	3	7	5

Table 3. Micoflora's influence on sunflower germination

Hybrid	**Germination (%)**	
	After 4 days	After 7 days
Performer	80	90
Favorit	85	95
NK Adagio	85	100
NK Meldimi	80	95

From our experiments, as it is shown in table 3, it was observed that after 7 days sunflower seeds germination was not affected for NK Adagio hybrid also was 95% for Favorit and NK Meldimi. The lowest seed germination rate was recorded for Performer hybrid seeds, respectively 90%.

CONCLUSIONS

The micoflora detected on the sunflower achenes was numerous, with pathogens belonging to *Alternaria*, *Penicillium*, *Stemphylium*, *Rhizopus*, *Aspergillus*, *Fusarium*, *Mucor*, *Sclerotinia*, *Trichoderma* and *Cladosporium* genera.

Alternaria spp. and *Stehphylium* spp. were the pathogens with the highest incidence rate on all analyzed hybrids.

Pathogen association did not affect the germination rate of seeds from the analyzed hybrids, except for Performer hybrid that presented the lowest germination rate of 90%.

LITERATURE

Afzal, R., Mughal, S.M., Munir, M., Sultana, K., Qureshi, R., Arshad, M. and Laghari, A.K. 2010. Mycoflora associated with seeds of different sunflower cultivars and its management. Pak. J. Bot.,42(1): 435-445

Beratlief C., H. Iliescu, 1997. "Highlights of proper sunflower seed storage", in: HELIA, 20. no. 26. pp. 121-132.

Cristea S., Jinga V., Zala C., Popescu C., Patrascu N., 2009. The oversee of mycotical rots and chemical influence on their attack on sunflower. Scientific Papers, Series A, Vol.LII.

Gain Report RO1508 of Global Agricultural Information Network - "Weather conditions impact Romanian oilseed crop", USDA Foreign Agricultural Service. 29.04.2015 http://gain.fas.usda.gov/).

Gheorghies C., Cristea S. 2001. Fitopatologie, vol.1, Ed. Ceres, Bucuresti.

Hulea A., 1969. Ghid pentru laboratoarele de micologie şi bacteriologie.

Kakde R. B. et al., 2012. "Storage mycoflora of oilseeds: a review", in: International Multidisciplinary Research Journal 2(3):39-42. ISSN: 2231-6302; Online: http://irjs.info/

Kiffer E., Morelet M., 2000. The Deuteromycetes. Mitosporic fungi, classifications and genera keys. Science Publisher Inc., Enfield, New Hampshire.

Mardare E.S., Cristea S., 2015. Researches on the reaction of sunflower hybrids to funal rot attack. Journal of Biotechnology supplement, Vol. 208.

Mardare E.S., Cristea S., Zala C., 2014. Researches on the micoflora of sunflower's achenes from hybrids cultivated in Fetesti area, Ialomita County. Scientific Papers, Agronomy, Vol.57 no.2, USAMV Iasi.

Nahar, S., Mushtaq, M. and Hashmi, M.H. 2005. Seed-borne mycoflora of sunflower (Helianthus annuus L.) Pak. J. Bot. 37(2): 451-457.

Varga M., Matei F., 2009. The application of statistical modelling tools growth evolution of food born moulds. Romanian Biotechnological Letters, vol.15,no.2, pp 4249-4259.

Zala C., Cristea S., Groza O., Cristea M., 2010. Pathogen fungi on the sunflower seed and their impact upon germination. Scientific Papers, UASVM Bucharest, Series A, Vol. LIII, pp. 454-459.

http://www.paginadeagricultura.ro/romania-pe-primul-loc-in-ue-la-productia-si-suprafata-cultivata-de-floarea-soarelui-in-2015/