



Mycoflora of maize seeds from Novi Pazar

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Abstract

Field and/or storage fungi may cause severe corn production losses. Laboratory experiments were set for fungal infection assessment of four maize hybrids: SY Ondina, Dekalb 370, Kneja 509 and ES Safari. Most present field (soil – borne) fungi found were from the genera *Alternaria*, *Mucor*, *Penicillium* and *Fusarium*. Plumules were most infected in Kneja 509 (50%) and least in SY Ondina (40%). Subepidermal, the seeds were infected with *Fusarium*, *Alternaria*, *Mucor*, *Penicillium*, *Helminthosporium*, *Aspergillus*, *Pythium*, *Rizocontia* and *Trichoderma*. The average seed contamination was highest in ES Safari (57%) and lowest in Dekalb 370 (16%). The genus *Trichoderma* was found only in SY Ondina (19%) and the hybrid Dekalb 370 (1%).

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Introduction

Good grain harvest can be guaranteed by healthy plant populations emerged from pathogens – free seeds. Seed infection can result in great yield losses (Chandra and Sarboy, 1997).

The most important fungal maize pathogens include species from the genera *Fusarium*, *Alternaria*, *Rhizoctonia*, *Penicillium*, and *Helminthosporium*. They infest the seeds and feed on their inner tissues (endosperm), which are rich in starch, leading to a low plant emergence rate. Fungi from *Pythium* genus damage the young seedlings, leading to root and emerging shoot decay in the initial stage of plant development (Chandrashekar, *et al.*, 2000).

During the storage, if seeds are contaminated with *Aspergillus* and *Penicillium*, toxic compounds may accumulate (aflatoxins and other cancerogenic substances) and depending on the level of infection maize quality may be reduced or even become hazardous (Christensen, 1974; Gachomo *et al.*, 2004; Iskender and Andrews, 2001). A significant amount of the world corn production is not safe for consumption due to mycotoxins present in the seeds (Janardhana *et al.*, 1999; Leukel and Martin, 2000; Sharma and Salunkhe, 1991). Another serious problem concerning maize industry is mold caused by the fungi, which lower corn quality and reduce its price (Girish *et al.*, 2004).

The purpose of the present study is to provide a sanitary fungal assessment and comparison of four maize hybrids (SY Ondina, Dekalb 370, Kneja 509 and ES Safari) provided by Terra LTD, Novi Pazar.

Materials and methods

Microflora of SY Ondina, Dekalb 370, Kneja 509 and Safari) was studied at the Laboratory of Phytopathology at The University of Shumen. The seeds were collected following the harvest in 2012 at Terra LTD, Novi Pazar. The collected seed samples were analyzed for the presence of seed mycoflora by employing standard blotter method and agar plate method (Geshele, 1971). For conducting incubation

tests sterile glass Petri plates of 9 cm diameter were used. The fungi detected were identified based on the cultural and morphological characteristics. In blotter method, the seeds were placed on three layers of moistened blotter papers in Petri plates. In agar plate method, the seed were placed over the surface of solidified Chapeks agar medium and Potato-Dextrose agar in Petri plates. In both the cases, the seeds were plated in Petri plates at the rate of 10 seeds/plate at equidistance and incubated in an incubator set to 24-25 ° temperature for seven days. The incubated seeds was observed on eighth day by using steriobinocular and compound microscope. The standard procedures of ISTA (ISTA 1976- Ivanov, 1980). Seeds were surface-sterilized with 1% sodium hypochlorite (NaOCl) for 4 min rinsed in sterile distilled water. Fungal isolation were obtained directly from fungal colonies by plating fungal spore or mycelium on Chapeks agar medium and PDA (Potato-Dextrose agar). Pure cultures of the fungal were transferred onto PDA slopes and stored at 24-25 ° C temperature.

The mycoflora associated with seed were identified using key given by Barnett (2003), Booth (1972).

The number of contaminated seeds was reported in percentage and the results were analyzed (Adegov and Dushenko, 1985).

Results and discussion

The seed colonies of fungal species established on the surface of are given in table 1. According to the data in the table on the surface of the studied maize hybrids seeds developed species of *Alternaria*, *Fusarium*, *Mucor* and *Penicillium* and colonies of the genus *Drechslera* are not found. The most powerful presence is recorded for the species *Penicillium*, *Fusarium* and *Mucor*. About the species of the genera *Penicillium* and *Mucor* to 100% presence in separate versions of the analysis is found up. The presence of species of *Fusarium* genus is found up to 50% on the surface of seeds of hybrid SY Ondina, ES Safari and Kneja 509. Most little participation have the species of *Alternaria* genus - it reaches 50% and only for the

standard variant in the analysis. There have been established differences in surface contamination of different hybrids seeds. This difference is in regard of quantity colonies and families fungi contaminated seeds surface. Most powerful presence of fungal colonies of all identified strains on seeds surface demonstrates the standard hybrid Kneja 509. It was

ascertained up to 100% participation of the species of *Penicillium* genera and *Mucor*, and up to 50% participation of species from *Alternaria* and *Fusarium* genera for this hybrid. Up to 50% of species of *Penicillium* and *Fusarium* genera and up to 25% presence of species of *Alternaria* genus in the seed surface of the hybrid ES Safary are ascertained.

Table 1. Surface fungal infection of maize seeds. Ranking: |+++| infection of 50 -100% of the seeds; |++| 25-50% infected; |+| 25% infected; |-| no infection.

(standard blotter method)

Hybrid	Seedling contamination (%)				
	<i>Alternaria</i>	<i>Mucor</i>	<i>Penicillium</i>	<i>Fusarium</i>	<i>Drechslera</i>
Fungi					
SY Ondina	+	+	++	++	-
Dekalb370	+	+	++	+	-
ES Safary	+	++	++	++	-
Kneja 509	++	+++	+++	++	-

Table 2. Subepidermal fungal infection of seeds. ($x=n \cdot 10^{-4}$ colonies/10g seeds; Sx =standard error; Sd =standard deviation; t =Student's t -distribution).

(Agar Plate method)

Fungi	Kneja 509 K		SY Ondina		Analyzed parameters			
	X	Sx	X	Sx	SD	D	t	Rank
<i>Alternaria</i>	6.1	4,87	7	2,55	5,49	54	1,83	0
<i>Fusarium</i>	3.5	4,76	0	0	4,67	35	1,45	0
<i>Helminthosporium</i>	3	1,7	7	2,55	3,06	4	1,3	0
<i>Mucor</i>	2	1,4	16	3,66	3,91	14	1,58	0
<i>Penicillium</i>	5	2,17	8	2,71	3,47	3	0,86	
P 5%>1,96	P 1%>2,58	P 0,1%>3,2						
	Kneja 509 K		Dekalb 370					
	X	Sx	X	Sx	SD	D	t	Rank
<i>Alternaria</i>	0	0	1.3	0,36	0,36	13	1,87	0
<i>Helminthosporium</i>	0	0	1.7	0,75	0,75	17	0,53	0
<i>Penicillium</i>	14	3,46	1.1	0,12	0,65	3	2,65	-
<i>Mucor</i>	1	0,99	1.9	0,92	0,92	18	0,59	0
P 5%>1,96	P 1%>2,58	P 0,1%>3,29						
	Kneja 509 K		ES Safary					
	X	Sx	X	Sx	SD	D	t	Rank
<i>Alternaria</i>	59	4,91	34	4,73	6,8	25	2,68	++
<i>Fusarium</i>	26	4,38	17	3,75	5,76	9	2,56	+
<i>Mucor</i>	6	2,37	30	4,58	5,16	24	1,65	-
P 5%>1,96	P 1%>2,58	P 0,1%>3,29						

There was found a smallest amount of species of established families on the seeds of hybrid Dekalb370. Most strong presence was reported for species of the *Penicillium* genus – up to 50% of the samples in the variant. Species of other genera are

present up to 25% of the samples in the variant. These results determine this hybrid as the least contaminated with fungi species isolated from the surface of seeds. The seeds surface of hybrid SY Ondina is close to the seeds of hybrid Dekalb370,

similar degree of infection with the fungal pathogens, as reported stronger presence of species of the genus *Fuzarium*- up to 50% of samples of the variant. These results were confirmed by the data obtained about % infected sprouts. They are represented in the last column of Table 1 and indicate that % contaminated sprouts is highest in the standard hybrid seeds Kneja 509- 70.0% and lowest in hybrid Dekalb370 seeds, which is recognized the least presence fungal types- 60.0%. Table 2 shows the results of the analysis to establish the internal contamination of the seeds of different maize hybrids. Isolated fungal species from the interior of the seed related to *Alternaria* and

Fuzarium genera as in a complete composition they are for the seeds of hybrid Kneja 509 and hybrid SY Ondina. But the number colonies for 10 grams of seeds of hybrid SY Ondina is shown smaller than the standard hybrid of Kneja 509. The internal contamination of Dekalb370 hybrids with species of *Alternaria*, *Helminthosporium* and *Mucor* genera is weaker than standard hybrid of Kneja 509. The data for internal mikoflora of hybrid ES Safary show that their internal infection with species of *Alternaria* and *Fuzarium* genera is higher than a standard hybrid of Kneja 509.

Table 3. Fungal infection of seeds (%).

(Agar Plate method)

Maize hybrid	Fungal infection by species (%)									Total infection (%)
	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Fusarium</i>	<i>Helminthosporium</i>	<i>Mucor</i>	<i>Penicillium</i>	<i>Pythium</i>	<i>Rhizoctonia</i>	<i>Trichoderma</i>	
ES Safary	31	0	35	3	2	5	1	8	0	57
SY Ondina	13	0	0	17	0	11	0	0	19	49
Dekalb 370	0	0	0	0	0	14	1	0	1	16
Kneja 509K	7	9	0	7	16	8	0	0	0	40

Table 3 shows the results of the analysis for total contamination of the seeds where is included microflora of damaged germs. In this analysis there are isolated species from another 4 fungi species - *Aspergillus*, *Pythium*, *Rhizoctonia* and *Trichoderma*, as *Pythium* *Rhizoctonia* are indicated as aggressive agent for cutting and causing root rot and *Trichoderma* as their antagonist (Ivanov, 1980). The analysis shows that the overall infection of the seed is highest for hybrid ES Safary - 57%, and lowest for хибрид Dekalb370- 16%. The hybrids SY Ondina and Kneja 509 are respectively with 49 % and 40% total seeds infection.

The method agar plate favour the growth of fungi and gives highest percent incidence due to PDA and Chapeks –Agar. The presence of fungi of the genera

Pythium and *Rizocontia* mikoflorata in the composition of the seeds, according to some authors entails the risk of rotting sprout and later by root rot (Christensen C., 1974).

The present findings of the seed borne fungal organisms were in agreement with the information of seed borne nature of the pathogen reported by Marley and Gbenga(2004).

Conclusions

Fungi from the genera *Alternaria*, *Mucor*, *Penicillium* and *Fusarium* were present on 50-100% of the seed coats from the studied hybrids. The average percentage of diseased seedlings was highest for the hybrid Kneja 509 (50%). The hybrid SY Ondina was least contaminated on the surface and least seedlings

were diseased (40%). The subepidermal test detected the genera *Fusarium*, *Alternaria*, *Mucor*, *Penicillium*, *Helminthosporium*, *Aspergillus*, *Pythium*, *Rizocontia* and *Trichoderma*. The hybrid Safari was most infected (57% total seed infection) while the hybrid Dekalb 370 was least infected (16%). The genus *Trichoderma* was found only in 19% of SY Ondina seeds and 1% of Dekalb 370 seeds.

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