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Molds in Grain Feeds

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Abstract

In agriculture and the production of grain feeds, in spite of all the measures of protection and control, the presence of molds in grains is almost unavoidable. Molds contaminate the animal feed and/or nutrients during the growth of plants, as well as in all the handling operations - during harvest, mowing, picking, transport and storage. The comparative research on presence and identification of molds, shown in this paper, was carried out in two phases, in 2011 and 2014, on the samples of grains, stored and intended for animal nutrition. In total, 72 samples of different grains were examined, most of them related to maize (56) from a domestic production in the Banja Luka region. Mycological analysis i.e. quantification and determination of fungi in all samples revealed dominant presence of *Fusarium* species in both phases. The occurrence of *Fusarium* species in 2011 and 2014, expressed as relative values, were 61.11% and 80.55%, respectively.

Key words: fungus, *Fusarium spp*, cereals

Introduction

Feed and/or nutrients are an ideal medium for the growth and reproduction of microorganisms, primarily fungi i.e. their filamentous forms.

Microscopic filamentous fungi described as molds among micro-organisms had for long been considered harmless, but due to their ability to produce toxic metabolites - mycotoxins, they were given special attention and importance (Waune, 2007).

Apart from their presence in cereals (corn, wheat, rice), molds are very often present in other agricultural crops and products (Logrieco et al., 2003).

However, unlike the field molds, whose survival under common conditions of grain storage is limited by time, the lifetime of storage molds on dried beans and the products of their processing is practically unlimited (Jemcev & Đukić, 2009). High humidity favors the fungi growth and mycotoxin production, and the temperature is often a key factor.

Materials and Methods

The research was conducted in two phases: the first phase in 2011 and the second one in 2014. During the study a total of 72 samples of different grains for animal feed were examined. Samples of maize (*Zea mays*) were dominantly represented as the most important nutrient for animal feed, in total of 54 samples (48 samples of corn grains and 6 samples of ground corn), 9 samples of rye (*Secale cereale*) and 9 samples of triticale (× *Triticosecale*).

Mycological techniques of isolation and identification of molds were used as indirect method of seeding on agar plates. For this purpose, two different selective media were used: Dichloran 18% glycerol (DG18) agar and Sabouraud dextrose agar (SDA). A series of dilutions was prepared from each of the sample. A volume of 0.1 ml from dilutions of 10^{-3} to 10^{-6} was inoculated onto each of the agar plates listed above in duplicate. Eventually, the plates were incubated on 27°C to 30°C during 1 week. Immediately upon completion of the incubation, colonies were determined up to genus level and counted. The number in all inocula was calculated and the results were expressed as colony forming units per gram (CFU/g) of sample.

Identification and determination of mold genera of isolated species were carried out on the basis of colony appearance and microscopic features according to standard identification keys (Jeršek, 2014, Pitt & Hocking, 2009; Mihajlović, 1983).

Results and Discussion

Insight into the presence and distribution of mold during both periods of research with the results of mycological analysis is shown in Tables 1 and 2.

Tab. 1. Mold contamination
Контаминација плеснима

Total number of fungi (CFU g-1 *) *Укупан број гљивица (CFU g-1 *)*	Phase I of research 2011 *Фаза истраживања 1, 2011*				Phase II of research 2014 *Фаза истраживања 2, 2014*			
	*n***	*%*	±	*Sp*	n**	%	±	*Sp*
Lack of growth *(без раста)*	5	13.88	±	5.76	0	-		
$< 10^3$	6	16.66	±	6.21	3	8.33	±	4.61
$10^3 - 10^4$	15	41.66	±	8.21	6	16.66	±	6.21
$10^4 - 10^5$	7	19.44	±	6.59	13	36.11	±	8.01
$10^5 - 10^6$	3	8.33	±	4.61	5	13.88	±	5.76
$> 10^6$	0	-			9	25.00	±	7.67

**Colony forming units per gram of sample* (*број колонија у 1 граму узорка*)
***Number of samples* (*број узорака*)

According to the results of mycological testing (Table 1), the degree of contamination in the first phase, in most samples (41.66%) was between 10^3 and 10^4 CFU/g, which was the highest relative value in 2011. However, there was no statistically significant difference between the samples and the level of contamination.

The total number of fungi is one of the indicators of the quality of nutrients used for production of compound feed for domestic animals. Corn samples from 2011 were relatively less contaminated than the samples from 2014. However, the marked difference was not statistically significant. In both periods, the results of mycological analyses of corn showed a wide range of mold contamination level. In the first stage, there was a lack of growth on 13.88% of the samples, with contamination of 10^5 to 10^6 CFU / g at 8.33%.

By examining data in Table 1, in the second phase in 2014, all samples were contaminated with molds. Moreover, 25% of the samples were found with extremely high contamination, more than 10^6, with a maximum contamination in samples of ground corn, where one of the samples showed high contamination of 8.4 x 10^6 CFU g^{-1}. In 2014, also, there was no significant difference in contamination of samples.

Table 2. Percentage of isolated *Fungi* genera in analyzed samples of cereals in 2011 and 2014
Проценат изолованих родова гљивица у анализираним узорцима жита у 2011. и 2014.

Genus *Род*	2011		2014		*t - тест*
	n*	% ± *Sp*	n*	%	
Fusarium spp.	22	61.11 ± 8.12	29	80.55 ± 6.59	1.859 ^{nz}
Penicillium spp.	5	13.88 ± 5.76	11	30.55 ± 7.68	1.736 ^{nz}
Aspergillus spp.	7	19.44 ± 6.59	13	36.11 ± 8.01	1.607 ^{nz}
Eurotium spp.	4	11.11 ± 5.23	12	33.33 ± 7.86	2.354 *
Alternaria spp.	5	13.88 ± 5.76	7	19.44 ± 6.59	0.365 ^{nz}
Rhisopus spp.	1	2.77 ± 2.74	8	22.22 ± 6.93	2.797**
Acremonium spp.	-	-	3	8.33 ± 4.61	-
Mucor spp.	2	5.55 ± 3.82	7	19.44 ± 6.59	1.823 ^{nz}
Paecilomyces spp.	-	-	1	2.77 ± 2.74	-
Cladosporium spp.	1	2.77 ± 2.74	-	-	-
Unidentified species	3	8.33 ± 4.61	6	16.66 ± 6.21	1.077 ^{nz}

***Number of samples (Број узорака)*

Data in Table 2 show that difference between the frequencies of genera occurrence was not statistically significant between the phases, except for genus *Eurotium* (statistically significant) and genera in *Rhizopus* and *Acremonium* (statistically highly significant).

By mycological analysis of samples of cereals, we isolated and identified 8 and 9 different genera of fungi in 2011 and 2014, respectively. As for the presence percentage in the analysed samples, genus *Fusarium* was found most frequently i.e. 61.11% and 80.55% in 2011 in 2014, respectively. Incidence of most of the identified genera was higher than the frequency of the fungi in 2011. In addition to *Fusarium* species in the samples of 2011, the most commonly identified fungi were genus *Penicillium* (13.88%), *Aspergillus* and *Rhizopus* (19.44%). In 2014, the mycological profile of samples of different cereals showed higher level of contamination compared to 2011.

Moreover, the genus *Fusarium* remains the dominant one in the second phase. However, other genera found dominant next to *Fusarium* are different than those in 2011, and are as follows (in descending order): *Aspergillus* (36.11%), *Eurotium* (33.33%) and *Penicillium* (30.55%). Except for minor differences in the frequency of the three most important toxigenic genera in both periods, increased occurrence of genera *Mucor* (19.44%) and *Rhizopus* (22.22%) was recorded in 2014, and those belong to a group of molds typical for an advanced decay of cereals stored in inadequate conditions.

In addition, Penčić and Lević (1994), summarizing the reports of almost a centenary-long research period, from 1906 to 1994, conducted in the former Yugoslavia, report that the most numerous species of fungi colonizing maize were species of genera *Fusarium*, and then *Penicillium* and *Aspergillus*.

Studies have shown that corn is on the first place among the cultivated crops world-wide, which *Fusarium* molds prefer as a suitable habitat and on which they are most commonly found. In addition to corn, *Fusarium* favors also wheat, rye and oats (Katalenić, 2004).

Krnjaja et al. (2010) quote their research according to which the most common molds were from genera *Fusarium* (56.09%), *Aspergillus* (54.35%), *Rhizopus* (40%), *Penicillium* (30.87%), *Mucor* (30.04%) and *Alternaria* (3.48%). The same authors (Krnjaja et al., 2010), quoted that in the samples of poultry feed from 2008, genus *Fusarium* was present predominantly with an incidence of 63.40%, which is consistent with our results in 2011, when the presence of *Fusarium* was recorded in 61.11% of samples.

Conclusion

Mycological testing on all samples of grains in both periods (2011 and 2014) found molds contamination in a wide range, with the highest number of samples (41.66%) contaminated in the range of 10^3 to 10^4.

In 2014, the samples were contaminated by 100%. However, there was no statistically significant difference in contamination between samples observed in 2011 and 2014.

By identification of isolates of molds, the dominant presence of the genus *Fusarium* was confirmed. In both periods, the presence of molds of genera *Penicillium*, *Aspergillus*, *Alternaria*, known as toxigenic species, was confirmed. At the same time, there were no significant statistical differences in the prevalence of these *Fungi* genera. A statistically significant difference was found in the mold of genus *Eurotium* and highly relevant difference within the genus *Rhizopus*, marked as species belonging to the group of advanced mold spoilage primarily indicating inadequate storage in the highly humid year of 2014.

References

Jemcev, V. & Đukić, A.D. (2009). *Mikrobiologija*. Novi Sad: Budućnost.

Jeršek, B. (2014). *Osnovni principi identifikacije plesni, kvasovk in bakterij v živilih*. Ljubljana: Biotehnička fakulteta, Oddelek za živilstvo, Katedra za živilsko mikrobiologijo.

Katalenić, M. (2004). Toksini *Fusarium* plesni i drugi toksini. *Meso-The First Croation meat journal*, *VI*(31-35), 55-60.

Krnjaja, V., Stojanović, Lj., Trenkovski, S., Bijelić, Z. & Tomašević, D. (2010). The frequency of pathogenic fungi genera in poultry feed. *Journal of Food, Agriculture & Environment*, *8*(3-4), 589-591. Retreived from: http://world-food.net/download/journals/2010-issue_3_4/54.pdf

Logrieco, A., Bottalico, A., Mule, G., Moretti, A. & Perone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. *European Journal of Plant Pathology*, *109*(7), 645-667.

Mihajlović, B. (1983). *Priručnik za identifikaciju bakterija, kvasaca i plesni*. Beograd: Savez veterinara i veterinarskih tehničara Jugoslavije.

Penčić, V. & Lević, J. (1994). Pregled identifikovanih gljiva na semenu i zrnu kukuruza u Jugoslaviji. *Selekcija i semenarstvo, 1*(1), 173-177.

Pitt J. I. & Hocking D. A. (2009). *Fungi and food spoilage*, 3rd ed. (pp 19-52). New York: Springer-Verlag.

Waune, L.B. (2007). Mycotoxins in the food chain: human health implication. *Asia Pacific journal of clinical nutrition*, *16*(1), 95-100.

Плесни у зрнастим хранивима за животиње

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Сажетак

У пољопривреди и производњи зрнастих хранива за животиње и поред свих мјера заштите и контроле практично је неизбјежно присуство плијесни на житарицама. Оне загађују сточну храну и/или хранива у току раста биљке и свих манипулативних радњи - током жетве, косидбе, убирања, транспорта и складиштења. Компаративна истраживања присуства и идентификације плесни, приказана у овом раду, спроведена су у двије фазе, 2011. године и 2014. године, на узорцима житарица складиштених и намјењених за исхрану животиња. Миколошки је претражено укупно 72 узорка различитих житарица, од којих се највише узорака односило на кукуруз (56), из домаће производње, са Бањалучке регије. Миколошка анализа, односно квантификација и детерминација гљивица у свим узорцима показала је доминантно присуство *Fusarium* врста у обе фазе истраживања. Доминација *Fusarium* врста 2011. године изражена као релативна вриједност, била је 61,11%, а 2014. године 80,55% заступљености.

Кључне ријечи: гљивице, *Fusarium spp*, житарице

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