**Streszczenie rozprawy doktorskiej**
**w języku angielskim**

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**Dissertation title:** Analysis of undesirable substances - secondary metabolites of moulds in plant raw materials and dietary supplements obtained from them

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The subject focuses on was dietary supplements, which are classified as foodstuffs under the law. Whereas, the production of medications is controlled and monitored by the Pharmaceutical Inspectorate, for dietary supplements the only form of control is to inform the GIS about the supplement being introduced to market. The notification does not require any information about the quality of the product or its raw materials. Also, supervision of dietary supplements already on the market is very limited, due to their excessive number. The lack of general rules and legal requirements concerning the quality of dietary supplements, as well as the lack of control at each stage of production, export and import, poses a serious threat to consumer health.

The following research objectives were distinguished in this study:
1.Identification of the risk associated with the possible presence of mould in samples of plant raw materials and dietary supplements;
2.Identification of the risk associated with the possible presence of selected mycotoxins and determination of their concentration levels in the tested material;
3.Estimation of human exposure to the presence of selected mycotoxins in the dietary supplements tested.

290 samples of dietary supplements based on plant materials, available commercially in Poland (pharmacies, online stores, stationary stores), were used for the study. The dietary supplements were based on fiber and psyllium seed husks, barley, milk thistle, hawthorn, red yeast rice, soybean, stevia, bee pollen, maca, and small-flowered willow.

Mycological analysis was performed on YGC medium (yeast extract, glucose with chloramphenicol). The results of the total number of moulds were expressed as the number of

colony forming units (cfu) per gram of sample. Identification of the fungal cultures was done to genus. Determination of mycotoxins (trichothecenes, zearalenone (ZEN), ochratoxin A (OTA), aflatoxin (AF), citrinin (CIT), patulin (PAT)) was performed using the HPLC-FLD and HPLC-MS/MS methods. Mycotoxin concentration values obtained during mycotoxicological analysis were used to estimate the exposure assessment, using a deterministic method.

Mycological analysis revealed that 243 samples out of the 290 were contaminated with mould (84%). The most contaminated group included dietary supplements based on small-flowered willow (100%) and bee pollen (95%). Moulds were identified in 93% of the samples of dietary supplements based on hawthorn and maca, and in 92% based on milk thistle. Red yeast rice was the least contaminated. The results showed that the total fungal count present in all samples was 5.2 x $10^6$ cfu/g, of which the total mould count in the material was 2.9 x $10^6$ cfu/g and the total yeast count was 1.7 x $10^6$ cfu/g. In all samples analyzed, the most common types of mould isolated from the test material were *Eurotium* spp. (35%), *Aspergillus* spp. (15%) and *Penicillium* spp. (13%).

Mycotoxicological analysis showed that 177 samples of the supplements analyzed were contaminated with mycotoxins, accounting for 61% of the samples. The most contaminated groups were milk thistle and soy-based dietary supplements (100%). The results showed that the most common mycotoxins that were present in the samples included PAT (52%) and ZEN (60%). T-2, DON and HT-2 were present at similar levels (39%, 34% and 33%). The content of OTA in all tested dietary supplements oscillated at the level of 16% and aflatoxin at 7%. CIT was absent in the tested material.

Positive samples of 8 of the 10 groups of supplements tested were used for analysis of toxin co-occurrence, because dietary supplements based on red yeast rice and hawthorn had been analyzed for a single mycotoxin. Only maca and green barley-based dietary supplements were not found to contain ≥ 6 mycotoxins. Of the other groups tested, milk thistle was contaminated with the highest number of toxins (≥ 6 mycotoxins = 73%). Co-occurrence of 5 mycotoxins at 11% was found in milk thistle and soybean, and in fiber and psyllium at 6%. In the other groups, no co-occurrence of 5 mycotoxins was found in the sample. In samples based on maca, bee pollen and stevia, the presence of 4 mycotoxins was not detected, in the other groups the co-occurrence ranged between 9-44%. Maca and small-flowered willow were contaminated with 3 toxins simultaneously, 60% and 59%, respectively. Each analyzed group of the material showed simultaneous contamination with at least 2 mycotoxins.

The results of the supplement analyses were used to attempt to assess consumer exposure to mycotoxins in the products. Of the groups considered, consumers of milk thistle were most

exposed to mycotoxins. A %TDI of 20.06% indicates chronic exposure to T-2 and HT-2. Higher %TDI values of these compounds were also found in small-flowered willow as well as fiber and psyllium. The highest consumer exposure to OTA was detected in maca, where the %TDI was 5%, and stevia (1.80%). Above 1% exposure to DON was also found in milk thistle (1.18%). For the other mycotoxins, the exposure rate was below 1%.

Overall, the results of the present study can serve as a guideline for farmers, private companies and, above all, for governmental authorities, which should monitor mould and mycotoxins levels in order to detect and eliminate potential risks as early as possible. Assessment of exposure to mycotoxins in dietary supplements may form the basis for EU legislation regarding their levels in dietary supplements.