TITLE: First Report of *Aspergillus flavus* on Organic Spelt Wheat in Serbia

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First Report of *Aspergillus flavus* on Organic Spelt Wheat in Serbia

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Spelt (*Triticum aestivum* ssp. *spelta* L. Thell) is an ancient wheat species of growing interest due to its pro-health properties and suitability for organic production. It is known as a rustic and disease-resistant plant. Hard adherent spelt hulls seem to pose an effective barrier for mycelial filaments of mycobiota thus protecting and reducing fungal and toxic contamination of spelt kernels (Vučković et al., 2013). *Aspergillus flavus* contamination has been rare in the agroecological conditions of cereal-growing areas of Serbia. However, increased temperatures and more frequent and persistent drought may favor *A. flavus* infection on cereal crops in temperate regions of Europe. The increasing importance of *Aspergillus* spp. relates to the toxicological properties of aflatoxins produced by the fungus, which are carcinogenic and teratogenic metabolons for both humans and animals (IARC, 2012). Spelt wheat spikes were sampled in 2016 from five genotypes grown organically in the region of Vojvodina, North Serbia. It was found that 8 to 10% of spelt spikes across the cultivars were shorter and dark-greenish with reduced number of small kernels, which appeared shriveled compared to healthy plants. From each sample, 100 symptomatic kernels were surface-disinfected in 0.4% NaOCl for 2 min, rinsed in sterilized water and cultivated on dichloran 18% glycerol agar

(DG 18). After incubation at 25°C for 7 days in darkness, 21 *A. flavus* isolates were obtained and cultivated on Czapek's agar (CZA) at 25°C for 7 days and *A. flavus* and *parasiticus* agar (AFPA) at 30°C for 3 days. The single-conidia isolates developed into yellow–green colonies with white mycelia at the edges, 65-70 mm in diameter after 7 days of growth in the dark at 25 °C on CZA. The biseriate conidial heads ranged in size from 400 to 800 μm and were finely rough-walled. Conidia were globose with relatively thin, finely or moderately roughened walls. After incubation in the dark for 3 days at 30˚C on *Aspergillus* differentiation agar – AFPA colonies developed orange colour on the reverse of the plate. Based on morphological and growth features, isolates were identified as *A. flavus* (Klich, 2002). Total genomic DNA was extracted from mycelia using the DNA Isolation Kit (Agilent Technologies, Santa Clara, CA). The rDNA-ITS region was amplified using the universal fungal primers ITS1 and ITS4 (White et al. 1990). The purified products were separately sequenced in both directions using the same primers. The ITS sequence (GenBank Accession No. KY038051) shared 100% identity with reference isolate *A. flavus* CBS 100927. The pathogenicity assay of identified fungi was performed by soaking surface sterilized spelt kernels in a conidial suspension (10^6 conidia/ml) and placing on 1% water agar (WA) plates and subsequently incubated at 28 ± 2 °C. The controls received only distilled water. The symptoms developed on inoculated kernels resembled those in the naturally infected kernels, while no visible symptoms were observed for negative controls. The pathogen was reisolated from the infected kernels and identified as *A. flavus*, fulfilling Koch's postulates. Although aflatoxins have been detected in spelt wheat products (Solarska et al., 2012), the presence of *A. flavus* in spelt wheat has not been reported previously. To the best of our knowledge, this is the first report of *A. flavus* detected on spelt wheat in Serbia. It would be instructive to further investigate the extent of occurrence of the fungus and associated mycotoxins that are of concern to food safety and quality.

51 References:

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53 IARC-International Agency for Research on Cancer. 2012. Monograph on the evaluation of
54 carcinogenic risk to humans. 1e104. http:/monographs.iarc.fr/ENG/Classification/index.php

55 Klich, M.A. 2002. Mycologia, 94:21.

56 Solarska, E., et al. 2012. J. Plant Prot. Res. 52:190.

57 Vučković, J., et al. 2013. J. Sci. Food Agric. 93:1996.

58 White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications.
59 Academic Press, San Diego, CA.