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Eco-friendly control of toxigenic *Fusarium avenaceum* on apple fruits

Mila Grahovac[1], Ferenc Bagi [1], Marta Loc[1], Mladen Petreš[1], Zagorka Savić[1], Dragana Budakov[1], Vera Stojšin[1], Ana Obradović[2], Slavica Stanković,[2] Jovana Hrustić[3]
[1]*University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia*
[2]*Maize Research Institute "Zemun Polje", Belgrade, Serbia*
[3]*Institut of Pesticides and Environmental Protection, Belgrade, Serbia*
Correspondence: mila@polj.uns.ac.rs

Introduction and context

In the last several years *Fusarium avenaceum* was recorded as the dominant causal agent of *Fusarium* rots of stored apple fruits. Besides economic losses measured by the number of decayed apple fruits, *F. avenaceum* is a significant pathogen also due its toxigenic potential. It can produce an array of different, highly toxic mycotoxins which can adversely affect human health.

Harmful effects of mycotoxin-contaminated food can be avoided by preventing infection by mycotoxin-producing fungi in field and during storage of apple fruits. Although available chemical protection technologies can significantly reduce decay and mycotoxin contamination, they usually do more harm than good (environmental pollution, toxic residues in food and soil, resistance occurrence etc.). Also, due to their ecotoxicological properties their use is limited only to pre-harvest application, while infections of apple fruits with *Fusarium* species occur also post harvest.

Due to high risk of soil, water, non-target vegetation contamination and presence of pesticide residues in food, interest in eco-friendly disease control alternatives, such as biocontrol agents, is increasing. However, the number of eco-friendly control tools on the market is insufficient to provide adequate substitution for chemical control measures. Moreover, it is desirable that biological control agent, if used as a living organism, is an autochthonous species in the region where it is intended for use.

Atoxigenic strains of *Aspergillus flavus* are considered as potentially efficient biocontrol agents, particularly against toxigenic strains of the same species. This study aims to determine the potential of atoxigenic strain of *A. flavus* as biocontrol agent against toxigenic *F. avenaceum* isolates on apple fruits.

Materials and approaches

The isolates of *F. avenaceum* used in the study originate from apple fruits and were obtained in the period from 2016 – 2018. The isolates were identified based on pathogenic, morphological and ecological properties, which was further confirmed by Polymerase Chain Reaction (PCR) using species-specific primers, and were proven to synthetize mycotoxins in cultivation medium as well as in apple fruits.

The atoxigenic strain of *A. flavus* (Figure 1) originates from the soil of the Republic of Serbia and was identified by PCR reaction using species-specific primers. By Cluster Amplification Pattern (CAP) it was determined that the strain is missing 40kb of the gene responsible for mycotoxin production (aflatoxin) and it was characterized as non-toxigenic.

As preliminary tests showed, in dual culture assay, *A. flavus* strain expressed competitive and antagonistic effects on *F. avenaceum* (Figure 2).

Pronounced competitive and antagonistic properties indicated the need for *in vivo* testing of the *A. flavus* strain on apple fruits. In *in vivo* trials apple fruits (cv. Golden delicious) treated with the *A. flavus* spore suspension (concentration 10^8 CFU/ml) immediately after harvest were artificially inoculated with toxigenic isolates of *F. avenaceum*. Developed rot lesions on treated and untreated fruits were measured and compared and it was recorded that atoxigenic *A. flavus* completely inhibits pathogenicity of toxigenic *F. avenaceum* on apple fruits (Figure 3).

Figure 1. Atoxigenic strain of *Aspergillus flavus*

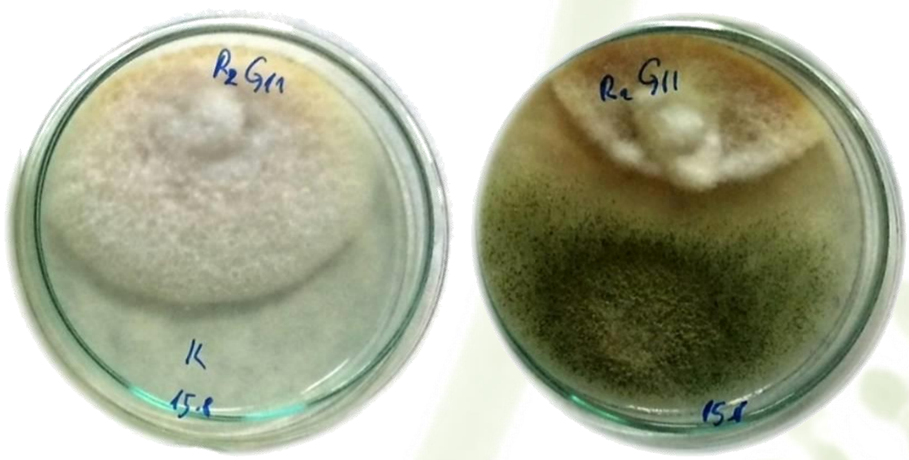

Figure 2. Inhibition effects on growth of *F. avenaceum* obtained by atoxigenic strain of *A. flavus*

Figure 3. Apple fruit rot caused by *F. avenaceum* on untreated apple fruit (left) and absence of rot development on *A. flavus*-treated apple fruit (right) – visible damage of fruits presents the site of artificial inoculation

Outcomes

The use of atoxigenic *A. flavus* as a control agent of toxigenic *F. avenaceum* results in healthy and safe apple fruits without pesticide residues or mycotoxin contamination. On the other side, considering the widespread of *A. flavus* in all natural environments and its origin, no harmful effects on the environment can be expected, while reduced use of pesticides for *F. avenaceum* control results in pesticide-free water, soil and apple fruits.

Transferability

The technology of apple fruit protection by the atoxigenic strain of *A. flavus* is easily tranferrable into practical conditions of apple fruit production. The spore suspension of the strain can be directly applied to apple fruits during vegetation and colonize apple fruits in the field, or can be applied as the dip-treatment prior to storage and in the process of fruit sorting.