Comparison between cultural methods and TLC for detection of atoxigenic strains of *Aspergillus flavus* isolated from Iranian pistachio

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Abstract

Aflatoxins (AF) are a group of carcinogenic mycotoxins produced by the same biochemical pathway in several *Aspergillus* spp. Different methods have been suggested to detect toxigenic and non-toxigenic strains such as cultural, analytical and molecular assays. The aim of this study was to compare cultural methods such as ultraviolet (UV) detection of AF diffused into coconut agar and colony color changing to pink when exposed to ammonia vapor to detect atoxigenic strians of *A. flavus* as well as with TLC. The isolates were collected from pistachio orchards in different agro-ecological zones of Iran. Two toxigenic and atoxigenic strains of *A. flavus* as positive and negative controls were used, respectively. The culture media were: potato dextrose agar (PDA), coconut agar medium (CAM) and yeast extract-sucrose (YES). The strains were inoculated at the center of solidified agar medium in 9-cm glass petri dishes and incubated at 25°C for 2 days. The color change of colony reverse was observed to detect toxigenic and non-toxigenic strains after exposing fungal colony for short time to a drop (0.2 ml) of 25% ammonia solution into the lid of the petri dish. The fungal colony was also exposed to the ultraviolet (UV) at 365 nm. The ability of the strains to produce aflatoxins was also assayed using TLC after culturing on rice powder for 10 days. The results showed that the colony reverse of AF-producing strains turned to pink and no change was observed with non-toxigenic strains. It should be mentioned the color change is restricted to the reverse of colony. The intensity of observed color was the same in CAM and YES media which was higher than PDA. The TLC and ammonia assays gave the same results to detect non-toxigenic strains. Using ammonia for the first screening atoxigenic strains of *A. flavus* is simple, reliable, fast and cost effective compared to the other cultural methods.