THE IN VITRO ANTAGONISTIC EFFECTS OF SOME BACILLUS SPP. ON THE GROWTH AND MYCOTOXIN PRODUCTION OF TOXIGENIC ASPERGILLI

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ABSTRACT

This study aimed to investigate the antagonistic effect of three strains of the genus Bacillus on toxigenic fungi contaminating food commodities, with emphasis on two ochratoxigenic isolates (A. ochraceus, A. westerdijkiae) and two aflatoxigenic isolates (A. flavus and A. parasiticus). In vitro studies were carried out using two different methods for cultivation tested bacilli with fungal isolates – coinoculation and dual culture method. The most sensitive isolate was A. ochraceus by both used methods. B. mycoides (5.72 ± 6.4 mm) and B. subtilis (5.08 ± 2.84 mm) were able to inhibit its growth and sporulation during ten days of cultivation (both inhibited the sporulation of A. ochraceus 100%) in coinoculation. The most effective in the dual culture method were B. subtilis and B. thuringiensis against A. ochraceus (growth inhibition rate 84.40%; 90.55%) and A. flavus (growth inhibition rate 91.54%; 92.43%). The most effective sporulation inhibitors were B. subtilis and B. thuringiensis, which completely inhibited the sporulation of A. ochraceus and A. parasiticus after ten days of coinoculation. Likewise, all tested bacterial strains showed complete inhibition of ochratoxin A synthesis in A. ochraceus and A. westerdijkiae exposure to bacterial volatiles. So, the current study illustrated that strains of the genus Bacillus could significantly inhibit the growth, sporulation, and mycotoxin production of toxigenic aspergilli and showing the great potential as a biocontrol agent of pre- and post-harvest food diseases caused by microscopic filamentous fungi.

Keywords: antagonistic activity, Bacillus spp., direct contact, mycotoxins, toxigenic aspergilli, volatiles

INTRODUCTION

Food spoilage, mainly microbial spoilage, is a major worldwide problem for the food industry, leading to the waste of edible foods and economic losses for producers and consumers (Lorenzo et al., 2018). Among microorganisms, filamentous fungi are the primary food contaminants, which can occur at various stages of the food chain (pre-/post-harvest, during processing, or storage) (Sadiq et al., 2019). The development of fungi or in food leads to changes in sensory properties of food, ranging from visual damage to noticeable changes in smell, taste, or texture, but can also have negative effects on human and animal health through the production of mycotoxins (Badiale Furlong et al., 2021). To prevent the deterioration of food crops and foodstuffs by fungal contaminants and their mycotoxins and thus prolong the shelf life of the products, different methods are used to eliminate them. This includes the use of fungicides, chemical preservatives, and various other chemical and physical methods. Despite their effectiveness, there are some limitations in using these methods due to their high cost, possible production of by-products with higher toxicity, loss of nutritional value of food (Karlovsky et al., 2016; Neme and Mohammed, 2017). In the view of the above context, there is a growing interest in the use of biological agents as preservatives in the field, processing, and postharvest handling has achieved the attention of the scientific community because they offer the best alternative to control fungi in food. The use of antagonist microorganisms or their secondary metabolites has proven to be safe and effective in the biological control of growth inhibition of many fungal pathogens (Ramos-Pereira et al., 2021; Zhang et al., 2021). The bacterial genus Bacillus is a promising candidate for a fungal antagonist in the sphere of bioprotection and bioconservation of food (Nath et al., 2015). The importance of this genus is associated with the biological control of numerous fungal pathogens contaminating many food commodities (Islam et al., 2019). There are several mechanisms for the use of Bacillus species to control food fungal pathogens, including competition in space or nutrients, antibiosis, and induced systemic plant resistance (Lastochkina et al., 2019). One of these mechanisms, antibiosis, has been reported as an essential mechanism by which the genus Bacillus produces active metabolites that inhibit fungal contaminants. Antifungal lipopeptides, volatile organic compounds produced by species of the genus Bacillus, are mainly biologically active substances used to inhibit fungal growth (Fira et al., 2018; Saleh and Jaoua, 2020). The role of Bacillus strains is not limited only to inhibiting fungal growth, but some species can also interact with fungal mycotoxins, resulting in their inactivation or removal through cell wall binding. These mechanisms, which help eliminate mycotoxins, include biodegradation of mycotoxins by chemical or enzymatic cleavages and adsorption, where the prominent role is played by the cell wall components of the microorganism (Chen et al., 2018). This study was designed to investigate in vitro inhibition effects of Bacillus spp. by using two different methods, coinoculation and dual culture method, against the growth, sporulation, and mycotoxin production of four toxigenic isolates of the genus Aspergillus (A. ochraceus, A. westerdijkiae, A. flavus, and A. parasiticus).

MATERIAL AND METHODS

Bacterial strains, identification, growth condition and inoculum preparation

Three strains of the genus Bacillus, namely B. subtilis, B. mycoides and B. thuringiensis were used in this study. The antagonistic bacteria of the genus Bacillus were obtained from food samples (B. mycoides and B. subtilis) and from soil samples (B. thuringiensis). Before identification, the strains were cultivated on nutrient broth (Inuva, Slovakia) at 30 °C for 24 h. Identification of the bacterial strains was performed by MALDI TOF MS (Bruker Daltonics, Germany, Maldi Biotyper) using single colonies of 24 h cultures of each strain according to Hlebova et al. (2017). To prepare a bacterial inoculum the bacterial strains were cultivated 24 hours at 30 °C in meat peptone broth (MPB) (HiMedia, India). Subsequently, the bacterial inoculum was prepared by diluting the bacterial suspension in sterile phosphate saline to a final concentration of 10⁶ cells/ml by adjusting the density to 0.5 McFarland units.
Fungal isolates, identification, growth condition and inoculum preparation

The following toxigenic isolates of fungi were used in this study: *Aspergillus flavus, A. parasiticus, A. ochraceus* and *A. westerdijkiae*. All the fungal isolates were obtained from green coffee beans samples, marketed in Slovakia. The isolates were identified to the species level according to morphological characteristics based on microscopy used follow diagnostic literature: Samson et al., 2002; Chench et al., 2017; Frisvad et al., 2019 and Yamairi et al., 2019. Aspergillus flavus isolates were tested for aflatoxin B1 and G1, and ochratoxin A, and their production was confirmed by thin layer chromatography (TLC) (Labuda and Tančínová, 2006). To prepare a fungal inoculum the isolates were cultivated on Sabouraud dextrose agar (SDA) (HiMedia, India) for 5 days at 25 °C. Then the inoculum was prepared according to Čisarová et al., (2020) to a final concentration 10⁵ CFU/ml by adjusting the density to 0.7-1.2 McFarland units, depending on the strain.

In vitro screening antagonistic activity of *Bacillus* spp. by volatiles

The antifungal activity of volatiles potentially produced by *Bacillus* species against toxigenic *Aspergillus* species (*Aspergillus flavus, A. parasiticus, A. ochraceus* and *A. westerdijkiae*) was tested using the coinoculation method (UI Hassan et al., 2019), with some modification (Fig. 1). A 100 μl of the prepared bacterial inoculum suspension (10⁵ cells/ml) was applied on MPA plates (Meat peptone agar; HiMedia, India) and cultivated without sealing for 24 hours at 30 °C. After 24 hours, the cover of the bacterial plate was replaced by a base SDA plate containing 5 μl of fungal inoculum (10⁵ CFU/ml). Subsequently, both parts of the plates were tightly sealed with parafilm to prevent volatiles escape. The plates were cultivated for 10 days at 30 °C in the dark. The plates were cultivated in an inverted position (the plate containing the bacterial inoculum was on top). The control treatment was represented by fungus inoculated plates without bacterial inoculum. The effects of bacterial volatiles on fungal growth and sporulation were evaluated by measured diameters (Ø mm) of the growing fungal colonies at the 2nd, 4th, 6th, 8th, and 10th day of cultivation with a digital caliper. All analyses were performed in 6 replicates. To evaluate the reversibility effects of bacteria volatiles on the fungal growth, on the 5th day of cultivation, a small piece of a fungal colony (Ø 10 mm) from the margin was removed with a sterile cork borer and transferred to fresh SDA plates. These plates were incubated for 10 days at 30 °C, and mycelial growth and sporulation were monitored (in the same way as before on 2nd, 4th, 6th, 8th and 10th day) and compared with the control group (plates with fungal inoculum never exposed to bacterial volatiles).

Antitoxigenic effect of *Bacillus* spp.

The antitoxigenic effect of the genus *Bacillus* was evaluated only after coinoculation method (including the reversibility test). For the detection of mycotoxins, the qualitative TLC (thin layer chromatography) screening method according to Labuda and Tančínová (2006) was used. After 10 days of cultivation (coinoculation and reversibility) the ability of the genus *Bacillus* to suppress the production of aflatoxin B1 (AFB1) in *A. flavus*, AFB1, and aflatoxin G1 (AFG1) in *A. parasiticus* and ochratoxin A (OTA) produced by *A. westerdijkiae* and *A. ochraceus* was evaluated. The used method is described in Čisarová et al., (2015). Visualization of mycotoxins AFB1, AFG1, ochratoxin A and their authentic standards (Sigma Aldrich, Germany) was performed directly under a UV lamp at 366 nm, where aflatoxin B1 and ochratoxin A appeared as a blue spot and AFG1 as a green spot.

Statistical analysis

Obtained results were evaluated by analysis of variance (one-way ANOVA) and Tukey HSD 95% multiple range test (*p<0.05*) using the program STATGRAPHICS Centurion XVI (version 16.1.11). The results of the percentage inhibition of spore germination and percentage of the mycelial growth inhibition (PMGI %) were calculated by using the MS Office Excel 2016 program.

RESULTS AND DISCUSSION

Antagonistic activity of the genus *Bacillus*

Contamination of food with various perishable microorganisms, mainly microscopic filamentous fungi, is a global problem that leads to large-scale economic losses. Nowadays, synthetic preservatives or antifungals are used to prevent their growth in food. However, these substances can affect humans and the environment, so more environmentally friendly alternatives are still being sought (Foydal et al., 2018; Morita et al., 2019; Zhang et al., 2020). Biological control of fungal growth and food deterioration by antagonist bacteria such as *Bacillus* spp. is an alternative to the chemical preservatives used. Many previous studies have reported that substances produced by some *Bacillus* species have antifungal activity against a broad range of fungi such as *Botrytis cinerea* (Toure et al., 2004), *Fusarium graminearum* (Wang et al., 2007), *Alternaria solani* (Zhang et al., 2020), including the genus *Aspergillus* (Jiang et al., 2014; Arfaoui et al., 2018). In this study, three strains of the genus *Bacillus* (*B. subtilis, B. mycoides, B. thuringiensis*) were assayed against four isolates of ochratoxigenic (*A. westerdijkiae* and *A. ochraceus*) and aflatoxigenic (*A. flavus* and *A. parasiticus*) aspergilli using two different methods. The first coinoculation method investigated the antifungal activities of potentially produced volatiles of the genus *Bacillus*. The second method was used to evaluate the antifungal activity of *Bacillus* spp. by the method of dual culture, where the tested bacilli were inoculated on one plate together with the fungi and were in contact with each other. Furthermore, there are many reports focused on the antitoxigenic potential of the genus *Bacillus* (Taniwaki et al., 2018; Ren et al., 2020; Higazy et al., 2021). So, the inhibition effect of tested bacilli on the production of mycotoxins was evaluated in this study, too.

Effect of *Bacillus* spp. on the growth and sporulation of tested fungi by bacterial volatiles

The result of the coinoculation method showed that in the presence of the *Bacillus* spp. and their released volatiles, the growth of tested fungi was inhibited significantly (*p<0.05*). The strains showed different antagonistic activity on mycelia growth and sporulation of toxigenic aspergilli. *A. ochraceus* was the most sensitive isolate from the tested ochratoxigenic fungi. *B. mycoides* (5.72 ± 6.4 mm) and *B. subtilis* (5.08 ± 2.84 mm) were able to inhibit vegetative growth and sporulation during ten days of cultivation (both inhibited the sporulation of A.)
ochraceus (100%) of this isolate significantly. After ten days of coincubation with these species, the radial growth of A. ochraceus resulted in smaller colony diameters compared to the control set (13.18 ± 7.07 mm). B. thuringiensis showed the ability to inhibit this species only until day 6 of cultivation (Fig. 1A) with the inhibition of sporulation 33.33%. The better results for B. thuringiensis were noted by Arfaoui et al. (2019). They found that B. thuringiensis 14F was able to inhibit 78% of A. ochraceus growth. Also, partial inhibition of A. westerdijkiae was found in treatment with B. thuringiensis (20.75 ± 12.44 mm) compared to a control set (23.65 ± 12.65 mm). However, B. thuringiensis did not show any inhibitory effect on A. westerdijkiae sporulation (Table 1). B. subtilis and B. mycoides were the most effective species in inhibiting the growth of this isolate. Antagonism of B. subtilis by volatiles was studied in work of Petchkongkaew et al. (2008). They found that the growth of A. westerdijkiae tested by the coincubation method for seven days was lower, about 34%. Inhibition of growth was associated with prolongation of the lag phase of the microscopic fungus, thereby reducing its ability to compete and utilize the nutrients in the environment. Also, Einloft et al. (2017) observed the inhibitory effect of B. saffensis RF69, B. amyloliquefaciens RP103, and B. subtilis RP242 on the growth of A. westerdijkiae as well as their ability to inhibit spore formation. They found that B. subtilis RP242 was the most effective biological control agent of fungal growth and sporulation. The results showed a 95.6% reduction in growth and spore germination after ten days of coincubation.

The inhibition activity of Bacillus spp. on the growth and sporulation of aflatoxicigenic aspergilli was much more potent, especially for A. flavus isolate. Results showed that among all tested toxigenic fungi of the genus Aspergillus, A. flavus was the most sensitive to the action of bacterial antagonists. The mycelial growth of A. flavus reached the smallest averages throughout all periods of cultivation (10 days), mainly in the presence of B. subtilis (1.60 ± 1.87 mm) and B. thuringiensis (0.85 ± 1.15 mm) (Fig. 2A). Nayak et al. (2020) tested the antifungal activity of 4 isolates of Bacillus spp. against the growth and sporulation of two strains of A. flavus (A2, A28). Their results showed that Bacillus vallismortis BC5 was the most potent in mycelial growth inhibition of A. flavus (92.3%), followed by B. subtilis BC6 (90%) and B. amyloliquefaciens BC1 and BC2 (88.6% and 89.3%). However, in our study, their efficacy did not correlate with inhibition of sporulation. B. subtilis showed no inhibitory effect on sporulation, and the effect of B. thuringiensis on A. flavus sporulation was minimal (16.67%) (Table 1). On the contrary, in their study, Gong et al. (2014) found the high inhibitory activity of B. subtilis on the sporulation of A. flavus. They found that bacillomycin D produced by B. subtilis fmi0 strain (CGMCC 0943) inhibited sporulation of A. flavus from 85.72% to 98.10%.

In this study, 100% inhibition of sporulation was observed in treatment with B. mycoides, but this species had only a mediatory inhibition effect on A. flavus growth after ten days of cultivation (18.72 ± 13.98 mm) in comparison with a control set (29.55 ± 10.20 mm). On the other hand, B. subtilis and B. thuringiensis in comparison to the control and A. flavus species had better inhibitory effects on the sporulation of A. parasiticus (100%), but these species inhibited its growth only moderately (Fig. 2B). The best inhibitory effect for A. parasiticus was found in treatment with B. mycoides (6.11 ± 3.78 mm) during the whole cultivation period (10 days). However, B. mycoides was not able to inhibit A. parasiticus sporulation (Table 1). Also, Chaves-Lopez et al. (2015) evaluated the antifungal activity of the volatile organic compounds (VOCs) produced by 75 different food-borne Bacillus species against Aspergillus niger Aspergillus flavus, Aspergillus parasiticus, Aspergillus clavatus, Fusarium oxysporum f. sp. lactucae and Monilophthora perniciosa. In their study, the species A. clavatus MG103 and A. parasiticus MG51 showed the least susceptibility. Similar to our study, the growth of these species was not affected by Bacillus spp. volatiles production.

**Figure 1** The antagonistic effect of the tested Bacillus spp. on the growth: A) A. ochraceus (n=6) and B) A. westerdijkiae (n=6) during 10 days of cultivation in the dark (in mm) at 30 °C

**Figure 2** The antagonistic effect of the tested Bacillus spp. on the growth: A) A. flavus (n=6) and B) A. parasiticus (n=6) during 10 days of cultivation in the dark (in mm) at 30 °C
Table 1 Inhibition of sporulation of tested aspergilli by Bacillus spp. after 10 days at 30 °C of coinoculation method

| Tested bacilli | Tested aspergilli | Inhibition of sporulation (%) |
|---------------|------------------|-------------------------------|
| *B. subtilis* | *A. ochraceus*    | 100.00                        |
| *B. mycoides* | *A. ochraceus*    | 100.00                        |
| *B. thuringiensis* | *A. flavus*     | 33.33                         |
| Control       |                  | 0.00                          |

Legend: --: no effect (without presence of Bacillus strains)

The cell wall of fungi is necessary for their homeostasis, and various environmental or antifungal stresses can cause modifications such as suppression of sporulation, insufficient development of hyphae, or pigmentation losses (García-Rubio et al., 2020). In this study, similar changes were observed. Compared to the control, some fungal isolates whose sporulation was partially or entirely suppressed showed an observed change or loss of mycelial pigmentation (Fig. 3).

After five days of coinoculation with individual species of bacilli, the cultured plates were opened, and one small pieces of fungal mycelia were removed and subsequently cultivated on fresh SDA plates for another ten days without the presence of tested bacteria. However, upon transfer to the fresh SDA media, in the absence of bacterial volatiles, all the fungal isolates showed normal growth and sporulation, comparable with control sets. Our results showed that the inhibition was poor and that the coinoculation of fungi with the volatiles of tested bacilli during the 5-days was insufficient for more significant inhibition of their growth and sporulation. For this reason, the obtained results are not presented in this work.

Similar results were obtained by U H Hassan et al. (2019) with Bacillus spp. against tested fungi and Fiori et al. (2014) who treated toxigenic *A. carbonarius* MPVA566 and *A. carbonarius* AN6 with antagonistic yeast *Candida friedrichii* 778. Also, in the study of Saleh et al. (2021), similar reversibility in mycelial growth and sporulation was observed in toxigenic fungi included *A. flavus* CECT, 2687 after removal from volatiles of *B. megaterium* BM344-1. So, these results suggested that the microbial volatiles effect was insufficient and that bacterial volatiles were continuously required to allow effective biocontrol of spoilage fungi.

Effect of Bacillus spp. on the growth of tested fungi by contact

In the dual culture method, only the effect of Bacillus species on the growth of toxigenic fungi was observed. The results of dual culture method showed that antifungal ability of bacilli had a certain inhibitory potential on the growth of all tested fungal isolates. Significant differences (p<0.05) between the antagonistic effect of tested bacilli were found (Table 2). *B. mycoides* showed the best inhibitory ability against toxigenic aspergilli.

Table 2 Antifungal activity (colony diameter in mm ± SD) of Bacillus spp. on the growth of tested aspergilli (n=6) by dual culture method, after 8 days of incubation in the dark at 30 °C

| Tested fungi                              | Bacillus subtilis | Bacillus thuringiensis | Bacillus mycoides | Control of tested fungi |
|-------------------------------------------|-------------------|------------------------|-------------------|------------------------|
| *Aspergillus ochraceus*                   | 3.59 ± 2.24a      | 2.17 ± 2.25a           | 3.92 ± 0.55a      | 23.00 ± 0.00a          |
| *Aspergillus westerdijkiae*               | 13.30 ± 1.59a     | 18.52 ± 0.97a          | 5.73 ± 5.34a      | 17.28 ± 0.28a          |
| *Aspergillus flavus*                      | 3.05 ± 3.07a      | 2.73 ± 4.04a           | 34.58 ± 1.34a     | 36.00 ± 0.00a          |
| *Aspergillus parasiticus*                 | 27.83 ± 11.70ab   | 36.00 ± 0.00b          | 21.34 ± 7.38b     | 36.00 ± 0.00b          |

Legend: a, b: data in the columns followed by the same letter are not significantly different in 95.0% Tukey HSD test, SD – standard deviation

*B. mycoides* showed the best inhibitory ability against ochratoxigenic aspergilli.

The percentage of mycelial growth inhibition (%) calculated after all days of cultivation (8 days) with comparison to control sets was 67.36 % for *A. westerdijkiae* and 82.96 % for *A. ochraceus* (Fig. 4). From the tested fungi, *A. ochraceus* was the most sensitive to the action of all *Bacillus* species (Fig. 5B). The most potent inhibition against this isolate exhibited *B. subtilis* (growth inhibition rate 91.54%) followed by *B. thuringiensis* (growth inhibition rate 90.55 %) and *B. mycoides* (growth inhibition rate 82.96%). The sensitivity of *A. flavus* isolate was lower compared to *A. ochraceus*, but its growth was significantly inhibited by *B. thuringiensis* (growth inhibition rate 92.43 %) and *B. subtilis* (growth inhibition rate 91.54%) compared to the control sets (Fig. 5A). Similar results with Bacillus subtilis against *S. rolfsii* obtained by authors Rajkumar et al. (2018) and Nayak et al. (2020) in their study found that Bacillus spp. were able to inhibit the growth of *A. flavus* up to more than 90%. A very poor inhibition effect was observed using the dual culture method with *B. westerdijkiae* and *B. subtilis*. *B. thuringiensis* inhibited the growth of this isolate only up to the sixth day of cultivation (Ø 8.97 mm) compared to control (Ø 13.71 mm). After the cultivation period (8 days), the diameters of *A. westerdijkiae* colonies were larger (18.52 ± 0.97 mm) than the colony diameters of control sets (17.28 ± 0.28 mm).

So, its growth was moderately stimulated by *B. thuringiensis*. 

Figure 3 Changes observed in mycelial pigmentation: A) normal fungal mycelium (never exposed to bacterial volatiles) (control); B) change of mycelial pigmentation after treatment with volatiles of tested bacilli

Figure 4 The percentage of mycelial growth inhibition PMGI (%) of the tested fungi by Bacillus species after 8 days of dual culture in the dark at 30 °C
Based on the results, can be concluded that the coinoculation method was better than dual culture method used. In the case of antifungalogenic aspergilli, the difference between two used methods in the action of Bacillus spp. were observed. Significant and stronger antagonistic effects of selected species of the genus Bacillus could be seen mainly in the case of A. flavus and A. parasiticus used coinoculation method in comparison with the dual culture method. The different effects of bacteria in the case of coinoculation method, which were resulted in a higher inhibition of fungal growth, may be due to the use of a more suitable culture medium for bacteria, and fungi, respectively. The more appropriate used medium in coinoculation method may have affected the potential production of Bacillus spp. volatile compounds, and since the bacteria did not come into direct contact with fungi, can be assume that the highest growth inhibition was due to their production. Also, Morita et al. (2019) studied the antifungal effect of Bacillus pumilus volatiles by cultivating fungi and bacteria on three different media: Trypt-Soya agar (TSA), Lauria-Betani agar (LBA) and TM Enterprise agar (TMES). They found that the bacterium grown showed the strongest inhibition effect against Penicillium italicum only on TMEA medium. Tye et al. (2017) report, that the volatile organic compounds produced by Bacillus spp. can easily evaporate and diffuse through pores filled with air and water. Due to these physicochemical properties, volatile substances are ideal candidates for cooperation or antagonism between soil microorganisms that do not live directly next to each other.

**Inhibitory effect of Bacillus spp. on mycotoxin production**

Bacillus spp. volatiles not only inhibited the vegetative growth but also affected the mycotoxin production of toxigenic fungi. Although some species of the genus Bacillus did not completely inhibit the growth of the tested fungi, but they were able to suppress the production of their mycotoxins, especially in the case of inhibition of ochratoxin a (OTA) production. All three bacterial isolates inhibited OTA production by A. westerdijkiae and A. ochraceus totally (100%) in comparison with control sets. Successful inhibition of mycotoxin biosynthesis was also observed in the case of reversible action of Bacillus spp., when 5 days exposure of microscopic fungi to bacterial volatiles was sufficient to completely inhibit their toxicogenic potential (Table 3). The effect of bacterial VOCs, specifically of B. licheniformis 350–2 on OTA synthesis was tested after the 10th day of coinoculation in study of Ul Hassan et al. (2019). Their results showed complete inhibition of OTA synthesis by toxigenic fungi A. westerdijkiae BA1, A. carbonarius MG7 and P verrucosum MC12. Nevertheless, A. ochraceus MD1 (21.84 µg/kg) and A. niger MC5 (29.32 µg/kg) were still able to produce OTA in the presence of bacterial volatiles, although the levels of OTA production were significantly lower than the levels of OTA production of the unexposed control (A. ochraceus MD1 - 87.21 ± 6.32 µg/kg, A. niger MC5– 75.44 ± 7.90 µg/kg). Einolf et al. (2017) also showed a reduction in the production of A. westerdijkiae OTA production by bacterial antagonists of B. safensis RF69, B. amyloliquefaciens RP103 and B. subtilis RP242 ranging from 62 to 96%. Some other authors have confirmed the inhibition of ochratoxin A by volatiles of Bacillus spp. (Jiang et al., 2017; Shukla et al., 2020; Higazy et al., 2021).

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