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SOME NATURAL AND SYNTHETIC COMPOUNDS INHIBITING THE BIOSYNTHESIS OF AFLATOXIN B1 AND MELANIN IN *Aspergillus flavus*

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Abstract

The control of ~~the~~ mycotoxin contamination of agricultural products represents a serious problem of the global food and feed industry. Aflatoxin B1 (AFB1) is one of the most dangerous mycotoxins due to its hepatotoxicity, carcinogenicity, and temperature resistance. Thus, the search for substances able to block its biosynthesis and, therefore, to prevent the toxin accumulation in food and feed is still relevant. This paper is devoted to the study of the ability of some natural and synthesized compounds to block the biosynthesis of AFB1 and/or melanin (both are secondary metabolites, which have common intermediates and common initial stages of the polyketide biosynthetic pathway) in toxigenic *Aspergillus flavus*. The studied compounds included lovastatin, and several commercial compounds: (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid, alafosfalin, (1-aminoethyl)phosphonic acid, and N-hydroxyputrescine. A mutant *Aspergillus terreus* strain 45-50 obtained earlier from the *A. terreus* ATCC 20542 was used as the lovastatin producer. N-hydroxyputrescine and some phosphoanalogues of amino acids were assessed by their influence on the pigmentation of fungal colonies grown on solid medium and on the specific AFB1 content in cultural broth determined after the cultivation of a fungus on liquid nutrition medium. According to the obtained results, the studied compounds were divided into three groups. To reveal changes in the colony morphology, toxigenic *A. flavus* strain AF11 was cultivated on agarized medium. Concentrations of tested compounds in the medium varied from 0.0001 to 0.1 % depending on their activity. To determine the effect of tested compounds on the AFB1 production, AF11 was cultivated for 170 h in a liquid Payne-Hagler medium. Solutions of the tested compounds were added to the medium up to the final concentration of 0.001-0.1 % (commercial compounds) or 0.0001 to 0.001 % (lovastatin). The efficiency of the AFB1 biosynthesis stimulation/inhibition was assessed by the comparison of its content in the cultural broth in the experimental and control (medium without additions) variants. In addition, the effect of lovastatin on the AFB1 accumulation in wheat grain contaminated with *A. flavus* AF11 was assessed. The performed screening allowed us to divide the studied compounds into three groups. The supplement of the nutrition medium with (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid, and alafosfalin caused a significant decrease in the AFB1 production, but did not influenced on the colony pigmentation. N-hydroxyputrescine and (1-aminoethyl)phosphonic acid were able to partially or completely block the melanin biosynthesis with the simultaneous increase in the AFB1 production. Lovastatin completely blocked both AFB1 and melanin production even at low concentrations (0.0005 %). Therefore, compounds from the first and second groups inhibit the AFB1 or melanin biosynthesis, respectively, via the blocking of stages located after the point of divergence of the corresponding biosynthetic pathways, whereas lovastatin either inhibits the polyketide biosynthesis before this divergence point, or simultaneously inhibits both AFB1 and melanin biosynthetic pathways after the divergence point. Thus, we first revealed the ability of lovastatin to efficiently inhibit the AFB1 biosynthesis and also to suppress the growth and development of a toxigenic *A. flavus*. We also showed that the treatment of wheat grain with lovastatin at 0.25 and 0.5 mg/g before the contamination of wheat with toxigenic *A. flavus* reduced the AFB1 accumulation in the grain 4-fold and 20-fold, respectively. Taking into account the non-toxicity of lovastatin and the possibility of its highly-productive microbiological synthesis, the further study of the revealed new property of this compound seems to be

very promising for a development of new antiaflatoxigenic preparations able to prevent the contamination of animal feed with AFB1.

Keywords: aflatoxin B1, fungal melanins, polyketides, biosynthesis inhibitors, statins, amino acid analogues

The control of a mycotoxin contamination of agricultural products represents a serious problem of the global food and feed industry. Aflatoxin B1 (AFB1) is one of the most dangerous mycotoxins due to its hepatotoxicity, carcinogenicity, and temperature resistance. Decontamination of toxin-contained substrates is an urgent problem for the agriculture and health [1]. There are at least two possible ways to reduce aflatoxin B1 contamination: the blocking of toxin biosynthesis or the degradation of already produced toxin [2].

Aflatoxins, which belong to the products of polyketide synthesis, do not possess any significant phytotoxicity. To date, there is no evidence of their role as fungal virulence factors [3, 4]. The ability to produce aflatoxins, probably does not play a vital role for *A. flavus*, but may give some advantage in competition with other microorganisms sensitive to aflatoxins [5].

Fungal aromatic polyketides, including aflatoxins, constitute a large group of biologically active compounds synthesized with the participation of enzymes belonging to the polyketide synthase (PKS) family [6]. Melanins of indole or pentaketid nature protect fungi against damaging biotic or abiotic factors [7-9]. In the genus *Aspergillus*, melanins, which represent hydrophobic macromolecular pigments, may be synthesized via two pathways depending on the fungus species; the first one is the indole pathway via DOPA (3,4-dihydroxy-phenylalanine), and the second one is the pentaketide pathway via DHN (1,8-dihydroxy-naphthalene) [10]. Melanins are required for the survival and virulence of many pathogens [11, 12]. For example, pathogenic micromycetes defective in melanin production are generally not able to survive in nature [13]. Their cell walls are much thinner than in the parent melanized strain [4], and their spores are unable to penetrate host plant tissues [14]. Moreover, a PKS-defective mutant of *A. flavus* formed unpigmented sclerotia and was significantly more sensitive to heat and ultraviolet radiation [15].

Structural similarities between intermediates of the melanin biosynthesis in *A. flavus* spores and norsolorinic acid, a stable intermediate of the aflatoxin biosynthesis, allow us to suggest the same precursors for aflatoxins and this pigment [16]. Probably, the AFB1 biosynthetic chain in *A. flavus* represents a branch of the main polyketide biosynthetic pathway, which results in the melanin production [17]. Therefore, a search for compounds able to block the polyketides biosynthesis prior to the branching point, i.e., simultaneously suppress both melanin and aflatoxin B1 production seems to be promising. Such inhibitors would be helpful for the development of preparations preventing the AFB1 accumulation in feed and food products, and for the reduction of the contamination of agricultural products with *A. flavus* because of the reduced viability of the melanin-deficient mycelium.

Earlier we have shown that some phosphoanalogues of natural amino acids and peptides can inhibit or stimulate toxin biosynthesis in phytopathogenic fungi [18]. We also found the ability of lovastatin, a polyketide biosynthesis product [19], to block melanin production in a phytopathogenic fungus *Pyricularia oryzae* [20]. It suggests that statins, like amino acid phosphoanalogues, may be considered as potential inhibitors of the polyketide biosynthesis.

In the present study, we first identified the relationship between the inhibitors of aflatoxin production and the suppression of the indole pathway of melanogenesis in *A. flavus*. We showed that derivatives of the natural amino acids are able to limit either aflatoxin B1 biosynthesis, or melanin production.

Moreover, we have first found that lovastatin might be used as a potential inhibitor of early stages of polyketide biosynthetic pathways, which simultaneously suppresses both melanin and aflatoxin B1 production, and demonstrated the reduction of the aflatoxin B1 accumulation in wheat grain as influenced by lovastatin.

The purpose of this study was the search of compounds blocking different stages of the aflatoxin B1 and melanin biosynthesis, as well as compounds able to simultaneously inhibit the biosynthesis of both metabolites. In addition, we studied the possibility to reduce the aflatoxin B1 contamination in grain infected by *Aspergillus flavus* and pre-treated with lovastatin, the most active inhibitor of both toxin and melanin production.

Tehnique. Aspergillus flavus AF11 (BKM F-27; All-Russian Collection of Microorganisms, G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia) was used as a toxigenic strain. Mutant strain *A. terreus* 45-50, obtained from *A. terreus* ATCC 20542 (American Type Culture Collection, Manassas, Virginia, США) was used as a lovastatin producer [21].

Among the potential inhibitors of poliketide biosynthetic pathways, we tested lovastatin and commercial preparations of (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid, alaphosphalin, (1-aminoethyl)phosphonic acid, and N-hydroxyputrescine commercial preparations. The cultivation of *A. terreus* 45-50 and isolation and purification of lovastatin were carried out as described earlier [21].

To reveal morphological changes in *A. flavus*, the fungus was cultivated on solid medium containing agar (20 g/l), east extract (5 g/l), glucose (20 g/l), and salt stock solution (100 μl/l of growth medium). The composition of the stock solution was as follows (per 100 ml distilled water): $FeSO_4 \cdot 7H_2O$ (2.4 g), $MnCl_2 \cdot 4H_2O$ (4.2 g), $CuCl_2 \cdot 2H_2O$ (1.2 g), $ZnSO_4 \cdot 7H_2O$ (8.4 g). Commercial preparations were tested at 0.001, 0.01 and 0.1 % concentrations, and lovastatin, as much more active AFB1 inhibitor, was used at 0.0001, 0.00025 and 0.0005 % concentrations.

To study the effects of tested compounds on the aflatoxin B1 production, AF11 was incubated in shake flasks for 170 hours at 27 °C in liquid Payne-Hagler medium [22] under aeration conditions using an orbital shaker Excella™ E-25/25R (New Brunswick Scientific Co., Inc., USA) at 200 rpm and 5-cm eccentricity. Prior to incubation, the tested compounds were sterilized by a filtration (Millipore filters, ø 0.25 μm), and added to the medium to the final concentrations. After fermentation, the AFB1 content in culture broth was quantified by high performance liquid chromatography (HPLC) as described earlier [2]. Stimulating or inhibiting effects on the AFB1 biosynthesis was evaluated as compared to control (incubation without tested substances). Mycelium was collected and, after the removal of the excess culture broth (CB), air-dried at room temperature up to a constant weight. The toxin-producing ability of the fungus was assessed by the AFB1 in CB (μg) normalized to the dry weight of mycelium (g).

The effect of lovastatin on the AFB1 accumulation in grain infected by *A. flavus* AF11 was evaluated in the following way. 20 g of wheat grain were placed into 250-ml flasks containing 10 ml of distilled water and autoclaved for 1 hour under a pressure of 0.5 atmosphere. Then 1 ml of a sterile lovastatin solution (1, 5, or 10 mg/ml) or sterile distilled water (control) was added into the flasks. After the mixing of the flask content, each flask was inoculated by 1 ml of the spore suspension of *A. flavus* (1×10^7 spores/ml) and mixed again. After the 8-day incubation at 26 °C, 50 ml of chloroform was added into each flask. Flasks were incubated for 3 hours at 26 °C on a New Brunswick™ Excella E25/25R incubation shaker (250 rpm, 5-cm eccentricity), then their content was centrifuged for 30 min at 8000 g at room temperature to separate grain from the

organic phase, and 200 μl of the chloroform extract was sampled from each flask. After the chloroform evaporation, the residue was dissolved in 200 μl of methanol. The AFB1 concentration was further determined by HPLC [2].

The statistical treatment of the obtained data was performed using STATISTICA 6.0 software. The significance of differences was confirmed by the *t*-test for independent samplings ($P \leq 0.05$). Y-error bars on the histograms indicate standard errors. All experiments were arranged in three replications, each variant included 5-6 replications.

Results. The list of commercial preparations tested as potential inhibitors of the polyketide biosynthesis is shown in Table 1.

1. Compounds tested as potential inhibitors of the polyketide biosynthesis

Compound	Formula	CAS	Manufacturer
(Aminoethyl)thiophosphonic acid	CH_3-$CH(NH_2)$-$P(S)(OH)_2$	188649-76-5	Angene International Ltd., China
(Aminomethyl)thiophosphonic acid	$CH_2(NH_2)$-$P(S)(OH)_2$	49592-64-5	Angene International Ltd., China
Alaphosphalin	CH_3-$CH(NH_2)$-CO-NH-$CH(CH_3)$-$P(O)(OH)_2$	60668-24-8	Sigma-Aldrich, USA
(1-Aminoethyl)phosphonic acid	CH_3-$CH(NH_2)$-$P(O)(OH)_2$	6323-97-3	TCI, Japan
N-hydroxyputrescine	NH_2-CH_2-CH_2-CH_2-CH_2-NHOH	6536-85-2	Angene International Ltd., China
Lovastatin	$C_{24}H_{36}O_5$ (gross-fromula)	75330-75-5	Own product of microbiological synthesis

N o t e. CAS — Chemical Abstracts Service Registry Number® (https://www.cas.org/content/chemical-substances).

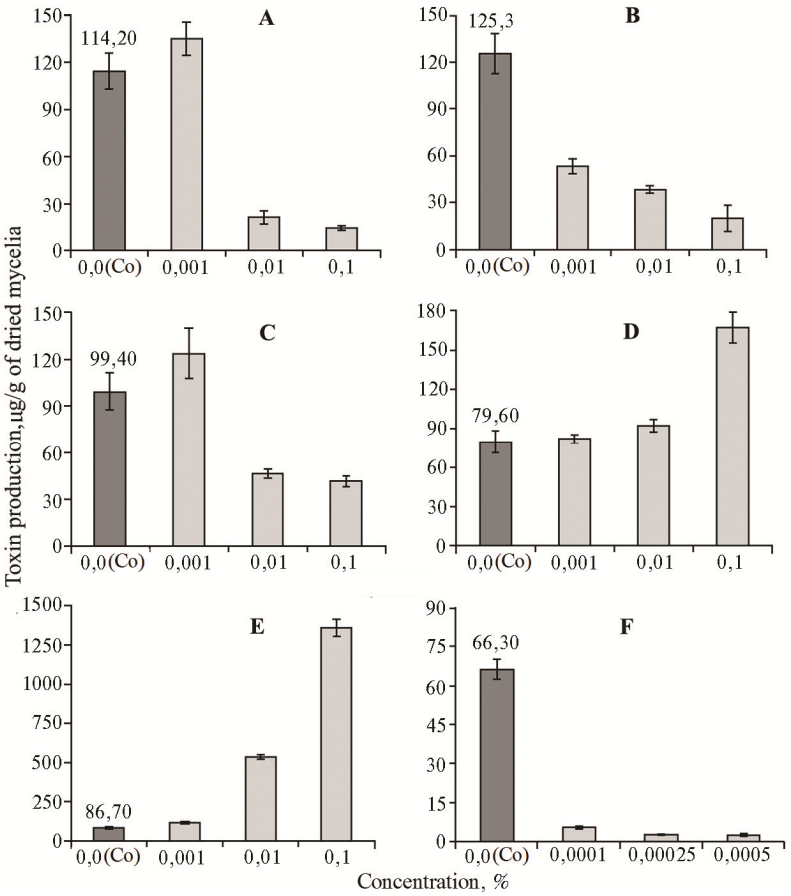

Fig. 1. Effect of tested putative inhibitors of the polyketide biosynthesis on the aflatoxin B1 (AFB1) **production in *Aspergillus flavus* AF11 in liquid medium:** A — (aminoethyl)thiophosphoric acid, B — (aminomethyl)thiophosphoric acid, C — alaphosphalin, D — (1-aminoethyl)phosphoric acid, E — N-hydroxyputrescine, F — lovastatin; Co — control.

The analysis of the AFB1 content in CB filtrates showed that the toxin production was noticeably influenced by all tested compounds (Fig. 1). It has been found that (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid, and alaphosphalin added to the medium at concentration equal to 0.01 % significantly reduced the AFB1 content in CB (Fig. 1, A, B, C). Among them, (aminomethyl)thiophosphonic acid was the most active inhibitor reducing the toxin production by about 50 % even at the concentration of 0.001 %.

One should mention that the differences in inhibiting effect between these three compounds at the effective concentration of 0.01 % were rather small (no more than 15 % on average). Probably, their targets were the same or neighboring stages of the polyketide biosynthesis pathway resulting in the AFB1 production. The inhibition of the AFB1 production was not accompanied by visible changes in the mycelium pigmentation in either liquid, or agar medium, i.e., these compounds, (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid, and alaphosphalin, have no apparent effect on the melanin production.

The addition of (1-aminoethyl)phosphonic acid and N-hydroxyputrescine stimulated the AFB1 production (see Fig. 1, D, E); the last compound was effective even at the concentration equal to 0.01 %, while the first one was active only at the highest level tested (0.1 %) doubling the AFB1 production as compared to control. The same level of N-hydroxyputrescine provided a 15-fold increase in the AFB1 production. The observed differences in AFB1 accumulation can probably be explained by the fact that these substances differ either in the targeted stages of the polyketide biosynthetic pathway, or in their inhibiting activity. In this, both agents suppressed mycelial pigmentation in liquid and on solid medium.

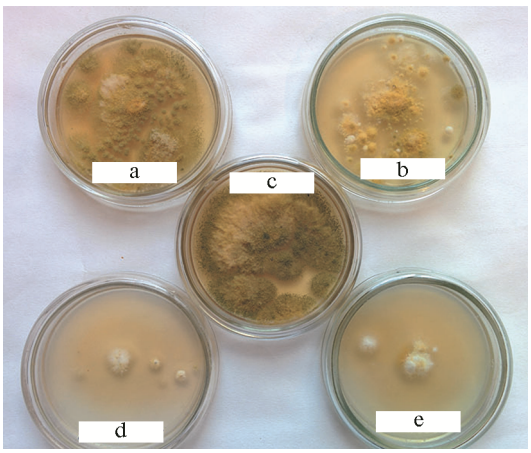

Fig. 2. Effect of different lovastatin concentrations on the mycelium pigmentation of *Aspergillus flavus* AF11: a — 0,001 %, b — 0,025 %, c — control, d — 0,01 %, e — 0,05 %.

Relatively low concentrations of lovastatin were able to suppress both AFB1 accumulation in CB (see Fig. 1, F) and mycelium pigmentation in either liquid and solid medium (Fig. 2).

In general, our data lead to the suggestion that the inhibition or stimulation of the AFB1 production by the compounds tested is caused by their ability to block different stages of the polyketide biosynthesis.

As we noted earlier, the biosynthesis of AFB1 and melanin has common initial stages; later the polyketide biosynthetic chain may branch in different pathways resulting in the production of either melanin, or AFB1. Stimulation or suppression of the toxin and(or) the pigment production depends on whether the polyketide synthesis is inhibited before or after the branching (Fig. 3).

The blocking of the biosynthetic chain at the stages located between the branching from a “main” pathway (melanin biosynthesis) and the final steps of AFB1 synthesis may result in the inhibition of toxin production, but did not affect the colony pigmentation that corresponds to the results obtained for three compounds of the first group — (aminoethyl)thiophosphonic acid, (aminomethyl)thiophosphonic acid and alaphosphalin. The blocking of the main biosyn-

thetic pathway between the branching point and the final steps of melanin biosynthesis may suppress the synthesis of the pigment in the later stages, with intermediates accumulated and used for the production of other polyketide metabolites. As a result, toxin production may increase (depending on the activity of the specific melanin inhibitor) that corresponds to the results for the second group of compounds, (1-aminoethyl)phosphonic acid and N-hydroxyputrescine.

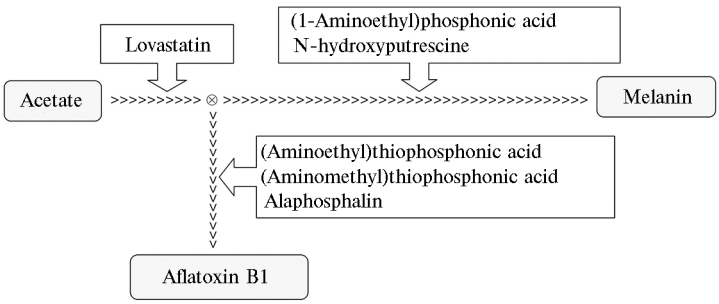

Fig. 3. Scheme of putative target points for natural and synthetic compounds blocking the polyketide condensation during melanogenesis and toxigenesis in *Aspergillus flavus*.

Finally, any suppression on the early stages of the main chain prior to branching should lead to a simultaneous suppression of both melanin and toxin production (see Fig. 3). In our experiments lovastatin inhibited production of both metabolites, that possibly suggests blocking the initial stage of the common biosynthetic pathway for AFB1 and melanin. Alternatively, one can suppose that lovastatin simultaneously inhibits both biosynthetic chains at the after-branching stages.

Natural compounds of plant origin, inhibiting the AFB1 biosynthesis were described in a number of publications [23, 24]. A melanin pigment from *Streptomyces torulosus* is known to inhibit the AFB1 biosynthesis in *A. flavus* [25]. Recently it was shown respiration inhibitors, such as rotenone, siccanin, and antimycin A, are able to significantly reduce aflatoxin production in *A. parasiticus* without affecting the rate of its growth on a solid medium [26]. It is also known that some inhibitors of the pentaketide melanin synthesis in fungi, such as tricyclazole, are able to suppress the AFB1 biosynthesis [27]. Presumably, these compounds inhibit reductase involved in the conversion of versicolorine A to dimethylsterigmatocystin at the later stages of the aflatoxin biosynthesis.

Our data confirming the relationship between AFB1 inhibitors and the suppression of the indolic pathway of melanogenesis in *A. flavus*, may be of interest to a wide range of researchers. In addition, the existing publications and patents do not contain any information about the ability of lovastatin to inhibit the biosynthesis of aflatoxins.

2. Aflatoxin B1 (AFB1) **accumulation and growth of *Aspergillus flavus* on wheat grain treated with various concentrations of lovastatin** (laboratory experiments)

Lovastatin, μg/g	AFB1, μg/g ($X \pm x$)	Mycelial growth and pigmentation
0	29.5 ± 2.63^a	Grain is completely covered by green mycelium
50	25.9 ± 1.49^a	Grain is completely covered by green mycelium
250	7.27 ± 1.08^b	Mycelial growth and pigmentation are partly suppressed
500	1.35 ± 0.14^c	Only some corns are covered by depigmented mycelium

N o t e. Differences between the values marked with different letters are significant at $P \leq 0.05$.

Lovastatin is nontoxic to warm-blooded animals [28-30] and can be microbiologically synthesized that makes its production to be relatively inexpensive. Since it was much more active towards the suppression of the AFB1 biosynthesis than other compounds studied, we investigated its effect on the AFB1 accumula-

tion in wheat grain artificially infected with toxin-producing *A. flavus*. After the treatment of grain with lovastatin at 0.25 mg/g, a significant (more than 4-fold) inhibition of AFB1 accumulation was observed (Table 2) with a simultaneous depigmentation of a fungal mycelium; in the case of a 0.5 mg/g dosage, more the 20-fold inhibition was shown. The further investigation of lovastatin properties may be promising for the development of preparations preventing the AFB1 accumulation in feed.

Thus, a relationship between the inhibitors of the AFB1 production and the suppression of the indolic melanogenesis in *A. flavus* has been revealed. We have shown some derivatives of natural amino acids are able to impair the AFB1 biosynthesis only, while other are able to suppress melanogenesis. Such inhibition of melanin generation was accompanied by a stimulation of the AFB1 production. It was found that lovastatin may be used as a potential inhibitor of both melanin and aflatoxin production. Probably, lovastatin action is targeted at a point located prior to the supposed branching of the common polyketide biosynthetic pathway into branches leading to the aflatoxin and melanin formation, i.e., at the early stages of the biosynthesis of these secondary metabolites. Alternatively, a simultaneous inhibition of both biosynthetic sub-pathways by lovastatin after the branching point could be assumed. We also showed that the treatment of wheat grain with lovastatin reduced the AFB1.

REFERENCES

1. Sun X., Sun C., Zhang X., Zhang H., Ji J., Liu Y., Tang L. Aflatoxin B1 decontamination by UV-mutated live and immobilized *Aspergillus niger*. *Food Control*, 2016, 61: 235-242 (doi: 10.1016/j.foodcont.2015.09.017).
2. Shcherbakova L.A., Statsyuk N.V., Mikityuk O.D., Nazarova N.A., Dzhavakhiya V.G. Aflatoxin B1 degradation by metabolites of *Phoma glomerata* PG41 isolated from natural substrate colonized by aflatoxigenic *Aspergillus flavus*. *Jundishapur Journal of Microbiology*, 2015, 8(1): e24324 (doi: 10.5812/jjm.24324).
3. McLean M., Watt M.P., Berjak P., Dutton M.F. Aflatoxin B1 — its effects on an in vitro plant system. *Food Additives & Contaminants*, 1995, 12: 435-443 (doi: 10.1080/02652039509374327).
4. Hasan H.A. Phytotoxicity of pathogenic fungi and their mycotoxins to cereal seedling viability. *Mycopathologia*, 1999, 14: 149-155 (doi: 10.1023/A:1007164617175).
5. Cotty P.J. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. *Phytopathology*, 1989, 79: 808-814 (doi: 10.1094/Phyto-79-808).
6. Crawford J.M., Townsend C.A. New insights into the formation of fungal aromatic polyketides. *Nat. Rev. Microbiol.*, 2010, 8: 879-889 (doi: 10.1038/nrmicro2465).
7. Belozerskaya T.A., Gesser N.N., Aver'yanov A.A. Melanin pigments of fungi. In: *Fungal methabolites*. J.-M. Merillon, K.G. Ramawat (eds.). Springer International Publishing, 2016: 1-29 (doi: 10.1007/978-3-319-19456-1_29-1).
8. Pihet M., Vandeputte P., Tronchin G., Renier G., Saulnier P., Georgeault S., Mallet R., Chabasse D., Symoens F., Bouchara J.P. Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. *BMC Microbiol.*, 2009, 9: 177 (doi: 10.1186/1471-2180-9-177).
9. Eisenman C., Casadevall A. Synthesis and assembly of fungal melanin. *Appl. Microbiol. Biotechnol.*, 2012, 93: 931-940 (doi: 10.1007/s00253-011-3777-2).
10. Pal A.K., Gajjar D.U., Vasavada A.R. DOPA and DHN pathway orchestrate melanin synthesis in *Aspergillus* species. *Med. Mycol.*, 2014, 52: 10-18 (doi: 10.3109/13693786.2013.826879).
11. Hamada T., Asanagi M., Satozawa T., Araki N., Banba S., Higashimura N., Akase T., Hirase K. Action mechanism of the novel rice blast fungicide tolprocarb distinct from that of conventional melanin biosynthesis inhibitors. *J. Pestic. Sci.*, 2014, 39(3): 152-158 (doi: 10.1584/jpestics.D14-033).
12. Takagaki M. Melanin biosynthesis inhibitors. In: *Fungicide resistance in plant pathogens: principles and a guide to practical management*. H. Ishii, D.W. Hollomon (eds.). Springer, 2015: 145-168 (doi: 10.1007/978-4-431-55642-8_11).
13. Dzhavakhiya V.G., Aver'yanov A.A., Minaev V.I., Ermolinskii B.S., Voinova T.M., Lapikova V.P., Petelina G.G., Vavilova N.A. *Zhurnal obshchei*

biologii, 1990, 51(4): 528-535 (in Russ.).
14. Lapikova V.P., Dzhavakhiya V.G. *Mikologiya i fitopatologiya*, 1987, 21(4): 358-365 (in Russ.).
15. Cary J.W., Harris-Coward P.Y., Ehrlich K.C., Di Mavungu J.D., Malysheva S.V., De Saeger S., Dowd P.F., Shantappa S., Martens S.L., Calvo A.M. Functional characterization of a veA-dependent polyketide synthase gene in *Aspergillus flavus* necessary for the synthesis of asparasone, a sclerotium-specific pigment. *Fungal Genet. Biol.*, 2014, 64: 25-35 (doi: 10.1016/j.fgb.2014.01.001).
16. Townsend C.A., Christensen S.B., Trautwein K. Hexanoate as a starter unit in polyketide biosynthesis. *J. Am. Chem. Soc.*, 1984, 106: 3868-3869 (doi: 10.1021/ja00325a031).
17. Butler M.J., Day A.W. Fungal melanins: a review. *Can. J. Microbiol.*, 1998, 44: 1115-1136 (doi: 10.1139/w98-119).
18. Khomutov R.M., Dzhavakhiya V.G., Khurs E.N., Osipova T.I., Shcherbakova L.A., Zhemchuzhina N.S., Mikityuk O.D., Nazarova T.A. *Doklady akademii nauk*, 2011, 436(4): 559-562 (in Russ.) (doi: 10.1134/S1607672911010078).
19. Yu J., Ehrlich K.C. Aflatoxin biosynthetic pathway and pathway genes. In: *Aflatoxins — biochemistry and molecular biology*. R.G. Guevara-Gonzalez (ed.). InTech, Rijeka, 2011: 41-66 (doi: 10.5772/23034).
20. Dzhavakhiya V.V., Petelina G.G. *Agro XXI*, 2008, 4-6: 33-35 (in Russ.).
21. Dzhavakhiya V.V., Voinova T.M. Optimization of fermentation conditions for high lovastatin producing mutant 45-50 of fungus *Aspergillus terreus*. In: *Biotechnology and industry*. G.E. Zaikov (ed.). NY, Nova Science Publisher Inc., 2004: 81-87.
22. Payne G.A., Hagler W.M. Effect of specific amino acids on growth and aflatoxin production by *Aspergillius parasiticus* and *Aspergillius flavus* in defined media. *Appl. Environ. Microbiol.*, 1983, 46(4): 805-812.
23. Zhou W., Hu L.B., Zhao Y., Wang M.Y., Zhang H., Mo H.Z. Inhibition of fungal aflatoxin B1 biosynthesis by diverse botanically-derived polyphenols. *Trop. J. Pharm. Res.*, 2015, 14(4): 605-609 (doi: 10.4314/tjpr.v14i4.7).
24. Holmes R.A., Boston R.S., Gary A., Payne G.A. Diverse inhibitors of aflatoxin biosynthesis. *Appl. Microbiol. Biotechnol.*, 2008, 78: 559-572 (doi: 10.1007/s00253-008-1362-0).
25. Shaaban M.T., El-Sabbagh S.M.M., Alam A. Studies on an actinomycete producing a melanin pigment inhibiting aflatoxin B1 production by *Aspergillus flavus*. *Life Sci. J.*, 2013, 10(1): 1437-1448 (doi: 10.7537/marslsj100113.214).
26. Sakuda S., Prabowo D.F., Takagi K., Shiomi K., Mori M., Omura S., Nagasawa H. Inhibitory effects of respiration inhibitors on aflatoxin production. *Toxins*, 2014, 6: 1193-1200 (doi: 10.3390/toxins6041193).
27. Wheeler M.N., Bhatnagar D., Rojas M.G. Chlobenthiazone and tricyclazole inhibition of aflatoxin biosynthesis by *Aspergillus flavus*. *Pestic. Biochem. Physiol.*, 1989, 35: 315-323 (doi: 10.1016/0048-3575(89)90092-8).
28. Wolin M.J., Miller T.L. Control of rumen methanogenesis by inhibiting the growth and activity of methanogens with hydroxymethylglutaryl-SCoA inhibitors. *International Congress Series*, 2006, 1293: 131-137 (doi: 10.1016/j.ics.2006.01.031).
29. Mori A.V., Mendonca C.X., Watanabe C. Effects of cholestyramine and lovastatin upon plasma lipids and egg yolk cholesterol levels of laying hens. *Brazilian Journal of Veterinary Research and Animal Science*, 2000, 37(1): 84-89 (doi: 10.1590/S1413-95962000000100015).
30. Kim J.H., Hong S.T., Lee H.S., Kim H.J. Oral administration of pravastatin reduces egg cholesterol but not plasma cholesterol in laying hens. *Poultry Sci.*, 2004, 83: 1539-1543 (doi: 10.1093/ps/83.9.1539).