

INFLUENCE ALKALOIDS FROM THE MARINE-DERIVED STRAIN OF THE FUNGUS *ASPERGILLUS FUMIGATUS* FRESEN. ON THE GROWTH OF SEEDLING ROOTS OF BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

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ABSTRACT

The effect of alkaloids fumitremorgin C (**1**), 12,13-dihydroxyfumitremorgin C (**2**), verruculogen (**3**), spirotryprostatin A (**4**), 6-methoxyspirotryprostatin B (**5**), spirotryprostatin F (**6**), fumiquinazoline C (**7**), bisdethiobis(methylthio)gliotoxin (**8**), and tryptoquivaline F (**9**) from the marine-derived strain of the fungus *Aspergillus fumigatus* on the growth of sprout roots of buckwheat at the concentration range 10^{-5} - 10^{-17} M was studied. It was shown that the alkaloids **1**, **3**, **4**, **6**, and **7** had a stimulatory effect on the growth of seedling roots of buckwheat. The stimulatory effect of compounds **3**, **4**, **6**, **7** was shown at ultra-low concentration (10^{-12} - 10^{-16} M). The doze-effect curve had a bimodal character.

Key words: *alkaloids, marine fungus Aspergillus fumigatus, buckwheat, doze-effect curve.*

1. INTRODUCTION

Marine-derived fungi are recognized as an important source of structurally novel and biologically active secondary metabolites [1-4]. Marine and terrestrial ecoforms of the fungus *Aspergillus fumigatus* are capable of producing compounds with an amazing variety of structures. Terpenoids, peptides, indole alkaloids, anthraquinones, and other compounds have been identified in its various extracts. The metabolites of this fungus exhibit antibacterial, fungicidal, insecticidal, cytotoxic and phyto-regulating activities [5, 6].

The function of alkaloids in plants are not fully understood. Probably, alkaloids are by-products of a metabolism in plants, or they are a reserve for the synthesis of proteins, chemical protection from animals, pathogens and insects, the regulators of physiological processes (growth, metabolism and reproduction), or the end products of detoxification [7-9].

In the scientific literature there were the works testified about positive effects of influence of ultra-low doses of biologically active substances on biological objects, in particular, on the growth of seedling roots of agricultural plants [6, 10-13].

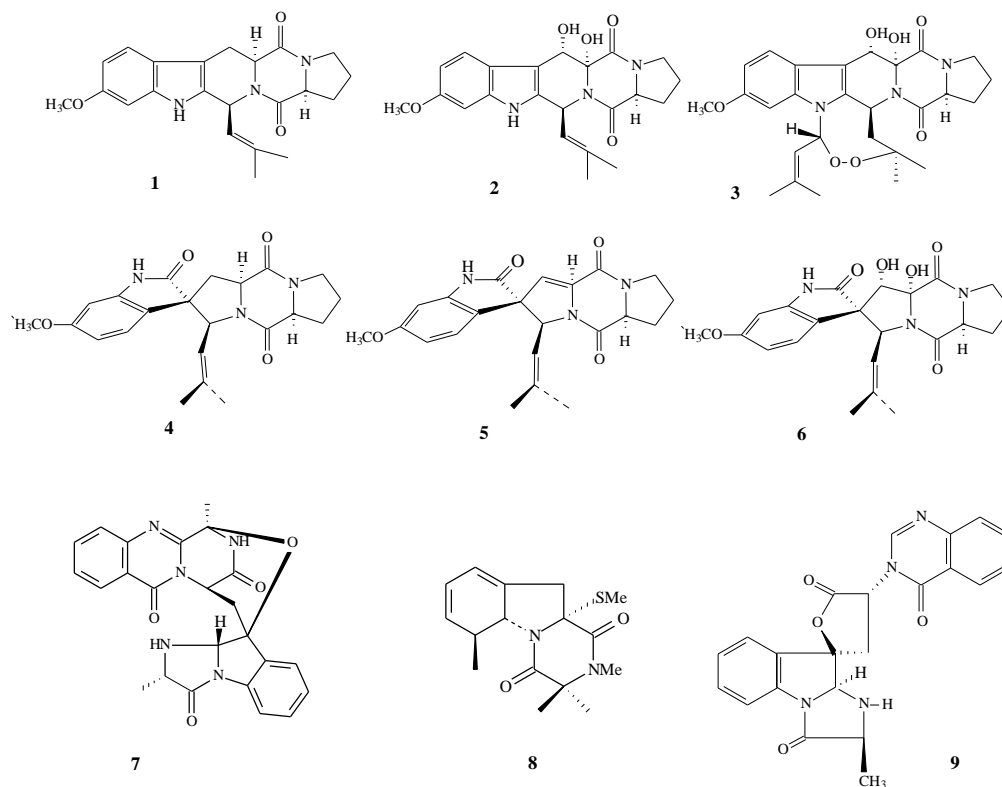
The purpose of the present work is to study the influence of alkaloids from the marine fungus *A. fumigatus* on the growth of seedling roots of buckwheat (*Fagopyrum esculentum* Moench) variety of Izumrud at low and ultra-low concentrations (10^{-5} - 10^{-17} M).

2. MATERIALS AND METHODS

The fungus was cultured for 21 days on rice medium specially modified by us [14]. The CHCl_3 -MeOH (2:1, v/v) extract of the culture of *A. fumigatus* was fractionated by Si gel column chromatography followed by normal-phase and reverse-phase HPLC to yield individual alkaloids **1-9**. Their structures have been confirmed on the basis of MS and NMR data [6, 15-17].

Seedling of seeds buckwheat (*Fagopyrum esculentum* Moench) of variety of Izumrud were object of study. Seeds from the 2011 harvest were obtained from Primorsky Research Institute of Agricultural, (Ussuriisk, Russia). We used a sprouting scheme in rolls of filter paper. Dry seeds were spread on strips of filter paper (12 x 42 cm) that were previously moistened with test solution, rolled, placed into beakers with a small amount of test solution (100 mL), and left for 3 days in a thermostat at 26-27 °C. The length of the main root of the seedling after incubation was measured. The controls were seedling of the same culture grown in distilled H_2O . The positive control was heteroauxin. Test results were estimated as the arithmetic mean of three repeated tests (20 seeds in each) and were expressed in percent of the controls ($M \pm se$). Results were processed statistically using the ORIGIN 7.0 computer

program. The significance of the results between control and test samples was estimated using the Student t-criterion ($p < 0.05$).



3. RESULTS AND DISCUSSION

Results show that alkaloids act on the shoots in different ways depending on the chemical structure (Fig.). Thus, fumitremorgin C (**1**) has shown stimulating effect on the growth of seedling roots of buckwheat at concentration 10^{-9} M (110 %). At the same time its dihydroxyderivative (**2**) showed slight inhibition of growth of seedling roots at concentrations 10^{-9} , 10^{-13} , 10^{-15} M in comparison with the control. Verruculogen (**3**) showed stimulating effect on the growth of seedling roots of buckwheat at concentrations 10^{-7} (121 %), 10^{-10} (116 %) and 10^{-16} (109 %) M. Spirotryprostatin A (**4**) rendered a positive effect on the growth of seedling roots of buckwheat at concentrations 10^{-7} (114 %) and 10^{-12} (113 %) M, while its dehydro derivative 6-methoxyspirotryprostatin B (**5**) has not shown stimulating effect. At the same time dihydroxy derivative **4**, spirotryprostatin F (**6**) showed the most pronounced stimulatory effect on the growth of seedling roots at concentration 10^{-10} (117%) M. Fumiquinazoline C (**7**) revealed two peaks of activity at concentrations 10^{-8} (113 %) and 10^{-15} (112 %) M, while the sulfur-containing alkaloid bisdethiobis(methylthio) gliotoxin (**8**) and tryptoquivaline F (**9**) were completely inactive compounds. Alkaloids **1**, **3**, **4**, **6**, and **7** are characterized by the fact that between the stimulating doses were found "dead spots". As a positive control was used heteroauxin (**10**), which showed both stimulating and inhibiting effects on the growth of seedling roots of buckwheat.

Earlier it has been shown that marine fungus *Acremonium striatisporum* produce diterpene glycosides of which virescensosides A, B, G and Q had a stimulatory effect on the growth of sprout roots of maize [11]. Later, from the marine fungus *Aspergillus fumigatus* were isolated alkaloids, which stimulated the growth of seedling roots of agricultural plants [6, 13]. The above-mentioned metabolites exerted a stimulating effect in ultra-low concentrations, while curves "dose-effect" had a bimodal character. The study of action of ultra-low doses of regulators of growth of plants is perspective, because it can be basis for new ways of application of biologically active substances in plant growing.

Thus, the marine fungus *Aspergillus fumigatus* is a producer of alkaloids **1-9**, which provided a varying degrees of stimulatory effect on the growth of seedling roots of buckwheat (*Fagopyrum esculentum* Moench) at concentrations 10^{-5} - 10^{-17} M. It was shown that the most effective stimulators of growth of seedling roots were alkaloids **1**, **3**, **4**, **6** и **7**. The stimulating effect of these substances has been shown in ultra-low concentrations (10^{-12} - 10^{-16} M). Alkaloids **1**, **3**, **4**, **6** и **7** can be recommended for study in the field conditions as growth factors buckwheat.

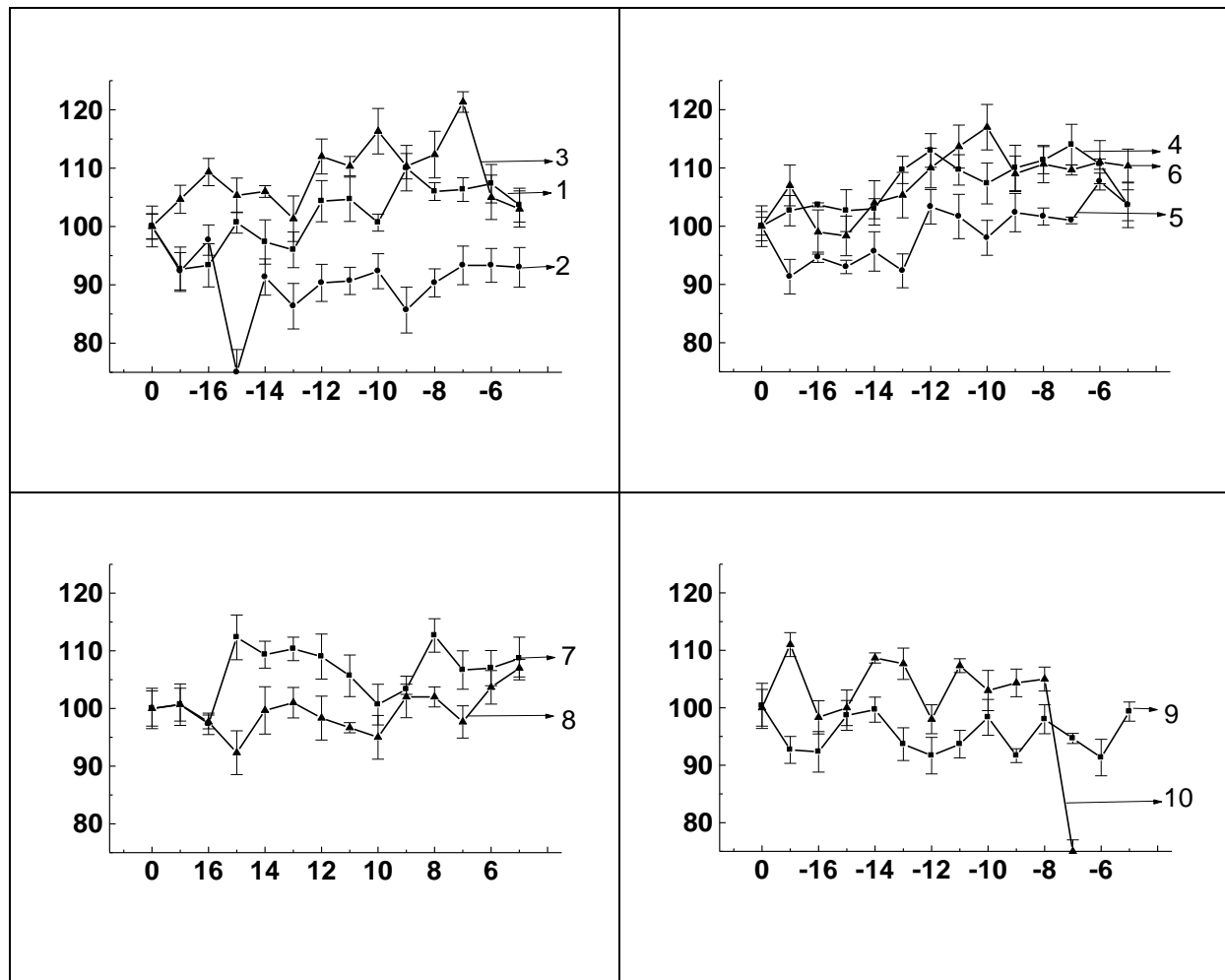


Figure. Influence alkaloids (1–9) from the marine fungus *Aspergillus fumigatus* and IAA (10) on the growth of seedling roots of buckwheat (*Fagopyrum esculentum* Moench). On the X-axis – lg molar concentration of biologically active substances; on Y-axis – length of roots, % of control.

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4. REFERENCES

- [1] P. Bhadury, B.T. Mohammad, P.C. Wright, The current status of natural products from marine fungi and their potential as anti-infective agents, *J. Ind. Microbiol. Biotechnol.* **33**, 325-337 (2006).
- [2] A. Debbab, A.H. Aly, W.H. Lin, P. Proksch, Bioactive compounds from marine bacteria and fungi, *Microbial Biotechnology* **3**, 544-563 (2010).
- [3] H. Greve, I.E. Mohamed, A. Pontius, S. Kehraus, H. Gross, G.M. König, Fungal metabolites: structural diversity as incentive for anticancer drug development, *Phytochem. Rev.* **9**, 537-545 (2010).
- [4] P. Proksch, A. Putz, S. Ortlepp, J. Kjer, M. Bayer, Bioactive natural products from marine sponges and fungal endophytes, *Phytochem. Rev.* **9**, 475-489 (2010).
- [5] J.C. Frisvad, C. Rank, K.F. Nielsen, T.O. Larsen, Metabolomic of *Aspergillus fumigatus*, *Med. Mycol.* **47**, Issue, 53-71 (2009).
- [6] Sh.Sh. Afiyatullo, O.I. Zhuravleva, E.L. Chaikina, M.M. Anisimov, A new spirotryprostatin from the marine isolate of the fungus *Aspergillus fumigatus*, *Chem. Nat. Comp.* **48**, Issue 1, 95-98 (2012a).
- [7] R.M. Kream, G.B. Stefano, Endogenous morphine and nitric oxide coupled regulation of mitochondrial processes, *Med. Sci. Monit.* **15**, 263-268 (2009).

- [8] Y.H. Liu, Z.S. Liang, J.L. Liu, Use of protocorm-like bodies for studying alkaloid metabolism in *Pinellia ternate*, *Plant Cell Tissue and Organ Culture* **100**, 83–89 (2010).
- [9] Q.F. Pan, Y. Chen, Q. Wang, F. Yuan, S.H. Xing, Y.S. Tian, J.Y. Zhao, X.F. Sun, K.X. Tang, Effect of plant growth regulators on the biosynthesis of vinblastine, vindoline and catharanthine in *Catharanthus roseus*, *Plant Growth Reg.* **60**, 133–141 (2010).
- [10] L.E. Makarova, G.B. Borovsky, A.M. Bulatova, M.G. Sokolova, M.G. Voronkov and A.N. Mirskova, Effect of Derivatives of Triethanolamine on the Root Growth of Monocotyledonous and Dicotyledonous Seedlings, *Agrochemistry Issue* **10**, 41–45 (2006) Rus.
- [11] M.M. Anisimov, E.L. Chaikina, Sh.Sh. Afiyatullof, T.A. Kuznetsova, Influence of diterpene glycosides from a sea mushroom *Acremonium striatisporum* on growth of roots of corn sprouts (*Zea mays* L.), *Agrochemistry Issue* 5, 34–38 (2010) Rus.
- [12] M.M. Anisimov, E.L. Chaikina, T.V. Malyarenko, A.A. Kicha, N.V. Ivanchina, Efficiency of the Steroid Glycosider from the Starfish *Asteropsis carinifera* and Heteroauxin to an Increase in the Sprouts of the Agricultural Plants, *Agrochemistry Issue* 3, 41–47 (2012) Rus.
- [13] Sh.Sh. Afiyatullof, E.L. Chaikina, N.A. Kraskovskaya, M.M. Anisimov, Effect of Alkaloids from the Marine-derived Strain of the Fungus *Aspergillus fumigatus* Fresen. on the Root Growth of Corn (*Zea mays* L.) Seedlings, *Agrochemistry*, Issue 7, 39–42 (2012b) Rus.
- [14] Sh.Sh. Afiyatullof, A.I. Kalinovskiy, T.A. Kuznetsova, V.V. Isakov, M.V. Pivkin, P.S. Dmitrenok, G.B. Elyakov, New Diterpene Glycosides of the fungus *Acremonium striatisporum* Isolated from a Sea Cucumber, *J. Nat. Prod.* **65**, 641-644 (2000).
- [15] Sh.Sh. Afiyatullof, A.I. Kalinovskiy, M.V. Pivkin, P.S. Dmitrenok, T.A. Kuznetsova, Fumitremorgins from the marine isolate of the fungus *Aspergillus fumigatus*, *Chem. Nat. Comp.* **40**, Issue 6, 615–617 (2004).
- [16] Sh.Sh. Afiyatullof, A.I. Kalinovskiy, M.V. Pivkin, P.S. Dmitrenok, T.A. Kuznetsova, Alkaloids from the marine isolate of the fungus *Aspergillus fumigatus*, *Chem. Nat. Comp.* **41**, Issue 2, 188–189 (2005).
- [17] Sh.Sh. Afiyatullof, O.I. Zhuravleva, A.S. Antonov, A.I. Kalinovskiy, M.V. Pivkin, E.S. Menchinskaya, D.L. Aminin, New metabolites from the marine-derived fungus *Aspergillus fumigatus*, *NPC* **7**, 497–500 (2012c).