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ABSTRACT BOOK and FINAL PROGRAMME

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MOLECULAR TEST SYSTEM FOR THE EXPRESS DETECTION OF HONEYBEE ASPERGILLOSIS

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INTRODUCTION:

Due to the peculiarities of the climate of central Russia (up to 6 months of winter) mycosis of honeybees cause serious damage to beekeeping. Among these diseases Ascospherosis and Aspergillosis are most often registered (1-4), and also called as chalkbrood and stonebrood, respectively. These two diseases have similar symptoms, however for treatment and prevention different fungicides are needed. The main etiological agents of stonebrood are fungi A.flavus, A.fumigatus and A.niger. The aim of this work was to create a diagnostic test system for indication of Aspergillosis pathogens using molecular genetics approach.

MATERIALS AND METHODS:

Pathogenic isolates of Aspergillus flavus, obtained from the aspergillosis infected apiaries of Republic of Tatarstan and collectible Aspergillus strains were used, such as: A.flavus (Link.), A.fumigatus (Fres.), A.niger (v. Tiegh), A.nidulans (Eidam, Wint.), A.terreus (Thom), A.ochraceus (Wilhelm), A.sulphureus (Fres. Thom et Church), A.candidus (Link), A.wentii (Wehmer), A.clevatus (Desm), A.giganteus (Wehmer). Isolation of DNA from fungal cultures and pathological test material was performed using affinity sorption on silica gel, which was preceded by lysing samples in guanidinium thiocyanate. In parallel investigation of pathological material by conventional microbiological methods conducted. Selecting and computer design of primers was performed on the basis of data on the sequence of microscopic fungi in the Genbank.

RESULTS:

The investigations conducted allowed to select two pairs of specific primers, while using one of them proved possible to conduct a rapid detection of microscopic fungus Aspergillus flavus in the cultures and the pathological material by PCR, and another pair was specific for the genome of the strain Aspergillus fumigatus (Fres.). A pair of primers AFL1-AFL2 identified specific fungal DNA from both collectible culture A.flavus and the material obtained from the apiary. The diagnosis of Aspergillosis caused by A. flavus was subsequently confirmed by a microbiological method. As a result of PCR reaction mixture, wherein the DNA A. flavus, isolated from pathological material, pure or mixed culture of the fungal specific fragment formed 250 bp presented, while in samples obtained from pure cultures of other fungi of the Aspergillus genus synthesis of the fragments was not observed. The second pair of primers, conventionally called AFU1-AFU2, identified specific DNA of the fungus A.fumigatus (Fres.) forming a fragment of 380 bp.

DISCUSSION AND CONCLUSIONS:

These results indicate that PCR with primers AFL1-AFL2 and AFU1-AFU2 is an effective test indicating major pathogens of bees Aspergillosis (A.flavus, A.fumigatus), allowing in a short time to establish the etiology of mycosis and to identify the type of pathogen.

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