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Ecological and Geographical Population of Genus Aspergillus Fungi

Gulnara Guliyeva[a]*, Alakbar Huseynzada[b], Abel Maharramov[c]

[a]*Republican Sanitary and Quarantine Inspection, Zargarpalan 121, Baku, AZ 1009, Azerbaijan*

[b,c]*Baku State University, Chemical faculty, Z. Khalilov 23, Baku, AZ 1148, Azerbaijan*

[a]*Email: laboratoriyarskm@rambler.ru*

[b]*Email: alekber-92@mail.ru*

Abstract

A numerous samples of plant origin (corn), canned baby food production and samples of coffee and tea were investigated due to the presence of various types of genus Aspergillus. During the analysis of secondary metabolites of investigated species, the indole containing alkaloids (clavine alkaloids, alpha cyclopiazonic acid (CPA) and diketopiperazine alkaloids) were revealed.

Keywords: Aflatoxin, Aspergillus; food products; indole containing alkaloids; ochratoxin A; toxicity.

1. Introduction

In these latter days food products of plant origin are imported from various countries. Many new genera of micromycetes, which have not previously been encountered in the conditions of Azerbaijan Republic, are imported with these products. Most of these isolated micromycetes were assigned to the genus of *Aspergillus* [1-3]. It is necessary to mention the following genera, which are actively producing toxic secondary metabolites, among isolated micromycetes: *A. ochraceus, A. flavus, A. fumigatus, A. penicilliodes* and *A. candidus* [4].

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* Corresponding author.

Among food products of plant origin, which were grown and refined in various ecological conditions over a period between 2015-2016 years, 58 genera of micromycetes, related to microspore and other systematic groups, were isolated and identified.

Fungi of *Aspergillus* genus have high metabolic activity and adaptation ability. Some representatives of the genus are conditionally pathogenic microorganisms and producers of some toxic compounds. First of all, aflatoxins (*A. flavus, A. parasiticus*) and ochratoxins (*A. ochraceus*) are the most spread mycotoxins of these fungi. The study of the mentioned mycotoxins and their producers, which are the natural contaminants of the food products, is related to the questions of environmental protection and human health. The problem of human mycotoxicosis is in the spotlight [5-7].

In last decades a lot of attention of doctors and mycologists is attracted by secondary mycoses, caused by potentially pathogenic (opportunistic) filamentous fungi, and the list of these fungi species, which are able to cause human mycoses, are permanently expanded [8-10].

In scientific studies researchers present data, which prove that the producing toxins, their quantity and differentiation may depend on geographic spreading of *Aspergillus* species, substrate isolation and kind of agricultural crop and production. Rarely, fungi of genus *Aspergillus* are mentioned in connection with alkaloids, although most of them have toxic effect on human organism.

Due to this fact, investigation of these metabolites and determination of synthetic ability of various species and isolates of *Aspergillus* genus are essential. According to above-mentioned, the infection control of imported products for the detection of the fungi of genus *Aspergillus*, using high sensitive and high specific test systems, was the target facility of our research [11].

2. Materials and methods

During the investigation, the collection of 34 isolates, belonging to various types of genus *Aspergillus* (*A. niger* (5 isolates), *A. fumigatus* (8 isolates), *A. flavus* (11 isolates) and *A. ochraceus* (10 isolates)), was created and isolated from plant origin products, manufactured in different countries.

For a period of 2015-2016 years were investigated 201 samples of plant origin (corn), 15 samples of canned baby food production, 10 samples of coffee and 24 samples of tea.

As a result of the monitoring of microscopic fungi quantity on the surface of maize, lentil, soybean and peas, it was found that their quantity varied between 0,001-30000 CFU/g.

The upper limit of the temperature optimum of development for such species as *A. fumigatus, A. niger* and *A. ochraceus*, belonging to the group of the most common causative agents of deep mycoses, is more than 37ºC (about 40 ºC). Microscopic fungi of *A. ochraceus* species were revealed in the samples of rye, sunflower and peas. Their quantity varied between 0,003-18000 CFU/g (figure 1).

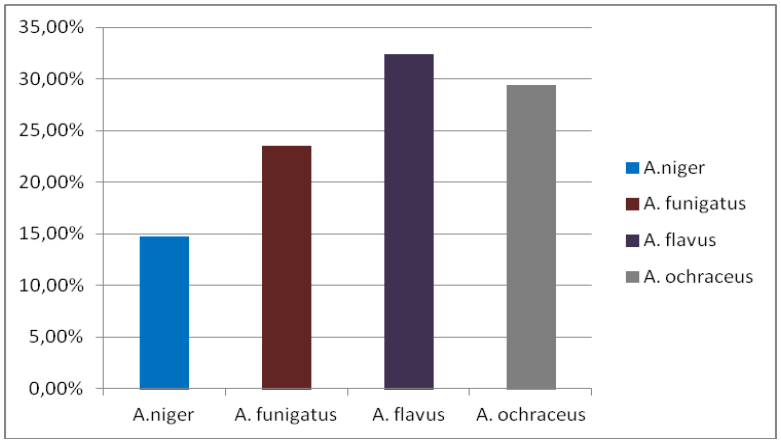

Figure 1: Content of *Aspergillus* isolates in food products

In the research were used modern express methods for the detection and counting of microorganisms: Compact Dry Yeast and Mold of German Company R-Biopharm (Darmstadt, Germany) [12].

Above-mentioned is achieved due to the presence of chromogenic enzyme substrate X-Phos in the composition of test system. Herewith, the growth of fluffy colonies of characteristic colour was observed, and the fact of growth inhibition of conditionally pathogenic fungi under the action of fungicides was revealed.

During investigation by classical methods, adopted in microbiology, the samples were chosen according to the growth and content of colony forming unit (CFU) on nutrient medium of leading companies BD and "Liofilchem" (Sabouraud agar, Potato Dextrose agar) and temperature control in the incubator at 25-28^{0}C by 5-14 days.

3. Results

During the analysis of secondary metabolites of investigated species, the main attention was paid to three groups of indole containing alkaloids: clavine alkaloids, alpha cyclopiazonic acid (CPA) and diketopiperazine alkaloids. Among the studied species clavine alkaloids were found in 3 out of 8 isolates of *A. Fumigatus*, which are 8,81% and are presented only by one metabolite, identified by us as fumigaclavine B. The other species of *Aspergillus* did not synthesize clavinove alkaloids.

CPA has attracted much attention due to toxicity and, as studies show, widespread presence among fungi of *Aspergillus* and *Penicillium* genera. This mycotoxin was found in 6 out of 10 isolates of *A. Ochraceus*, extracted from tea of various sorts and manufacturer countries, and are 17,64%. Our observation in this research demonstrates that fruit tea with various additions increases the content of ochratoxin to 2-12%; green tea - to 10-24% and black baikhovi – to 7-59% per day from toxicity threshold. If ochratoxin has not been found, it does not mean that this product is non-toxic, it is necessary to consider the presence of ochratoxin derivatives and product metabolites.

Indole containing diketopiperazine alkaloids are widespread among fungi of *Aspergillus* genus. Metabolites of

this class were found among the isolates of *A. Fumigatus*, *A. Niger*, *A. Flavus* and *A. Ochraceus*. They were found in 2 out of 5 isolates of *A. Niger*, in 7 out of 11 isolates of *A. Flavus*, in 3 out of 8 isolates of *A. Fumigatus*, in 2 out 10 isolates of *A. Ochraceus*, which are totally 41,17%.

4. Discussion

Due to the obtained results, it can be concluded that 67,62% of investigated samples were polluted by the fungi of *Aspergillus* genus (Figure 2).

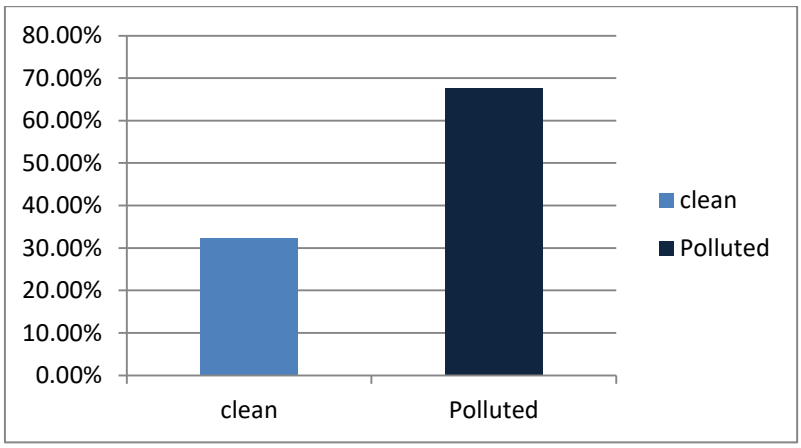

Figure 2: Quantity of polluted food products

Most of the isolates of A. flavus and A. ochraceus produced indole containing alkaloids, many of which were dangerous mycotoxins. All these species of Aspergillus are the most dangerous contaminants of food products (Figure 3).

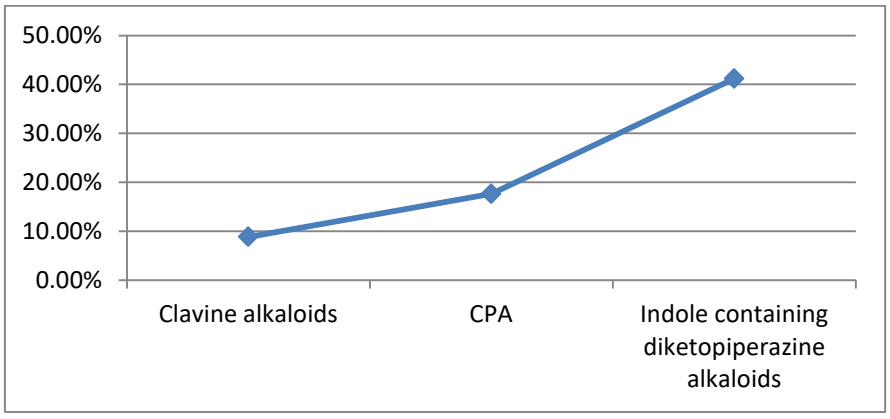

Figure 3: Content of indole alkaloids in food products

Consequently, the widespread of fungi of *Aspergillus* genus on the grains of various crops, coffee and tea causes accumulation of aflatoxins and ochratoxins, which is able to have a negative effect on quality of food products.

The crucial ecological factors, which are necessary for the development of mycroscopic fungi, are the content of

organic compounds, moisture, temperature and pH of their habitat.

In connection to the above-mentioned, the conducting of thorough mycological and mycotoxycological control of agricultural, livestock and food products should be considered foreground issue, and the problem of ecological and geographical population of toxigenic fungi of *Aspergillus* genus should be considered actual. It is necessary to do the screening study of biological activity of various chemical compounds toward fungi of *Aspergillus* genus, and also their secondary metabolites.

4. Conclusion

Therefore, the problem of contamination of the food is extremely urgent and is the main part of the global problem of the biosphere pollution. A leading role among the fungi that produce mycotoxins is given to micromycetes of *Aspergillus* genus. Our research led to the conclusion that 67,62% of investigated samples were polluted by the fungi of *Aspergillus* genus. Due to the fact that the toxic effects of mycotoxins on humans are still poorly understood, it is highly required to investigate the influence of enterotoxic metabolites of *A.flavus, A niger* and *A ochraceus*.

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