RESEARCH ARTICLE

The Production of Aflatoxins and Ochratoxin-A by Aspergillus Strains Isolated from Rice: Under In vitro Conditions

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Abstract:

Background: Rice supplies a significant proportion of nutritional needs around the world. The fungal species that cause severe contamination of rice grains have created a major challenge to ensuring food safety.

Methods: Thus, this study adopts an appropriate local method using potato dextrose agar (PDA) and thin-layer chromatography (TLC) for the production of Aflatoxins and Ochratoxin-A in Aspergillus flavus (NRRL strain 2999) and A. acrasus (NRRL strain 7431), respectively.

Results: Promising early results suggest that an optimum protocol for the production of mycotoxin includes a temperature of 28°C for 21 d incubation. The average levels of A. flavus and A. acrasus were 625 and 482.67 μg/g, respectively, by comparing the fluorescence with the standard. As a result, a new and rapid method using PDA as a culture medium and TCL was developed to produce mycotoxins in rice from the Persian market.

Conclusion: This study provides a novel (optimum) mechanistic approach concerning mycotoxins production from fungal species that could improve quality and ascertain its safety either in the field or in storage.

Keywords: Mycotoxin, Production, Culture media, Thin-layer chromatography, Persian market, Aflatoxin, Ochratoxin-A.

1. INTRODUCTION

Rice (Oryza sativa), being the second most consumed cereal worldwide, constitutes an important source of potential contamination by fungal pathogens [1, 2], and mycotoxin pollution is widespread in this strategic food.

Mycotoxins are secondary fungus metabolites that induce chronic and acute toxic effects in animals and humans [3], with physiological, biochemical, and pathological changes by disrupting nutrient digestion, absorption, and metabolism [4]. Meanwhile, Aflatoxins (AFs) and Ochratoxin A (OTA) produced by Aspergillus strains are extremely carcinogenic mycotoxins [5], which are capable of causing mycotoxicosis (disease and death) [6]. During the last decades, the AFs and OTA are mycotoxins of major significance and hence there has been important research on a broad range of analytical and detection approaches that could be practically and useful [7]. These toxins have attracted great concern due to their adverse effects and agroeconomic significance [8]. Thus, the reduction of mycotoxin contamination in rice as well as estimating average dietary exposure seems to be necessary.

Various reputable Persian articles from different publishers as reference sources reported that some of the rice spices in the local market are imported from abroad and thus could be contaminated with fungal species [9 - 11]. On the other hand, a variety of analytical ways have been used to determine harmful fungi and the production of mycotoxins in rice. However, AFs and OTA have been detected in rice, but there is no valid published data to achieve an appropriate method for the production of these toxins under in vitro conditions. This study provides information about mycotoxin production in rice, thus could lead to improved quality and ascertaining its safety.
2. MATERIALS AND METHODS

Aspergillus strains used in this study were acquired from the India Chandigarh Gene Bank for the production of AFs and OTA in A. flavus (NRRL strain 2999) and A. acrasus (NRRL strain 7431) in rice, receptively.

2.1. Preparation of Culture Media

To culture Aspergillus strains, a new (optimum) method using potato dextrose agar (PDA) as a culture medium was developed. First, we poured 9.75 g amount of the PDA culture medium (Potato Dextrose Agar) inside an Arlan, and mixed it with a certain amount of distilled water on the flame until the solution color became clear and reached the boiling temperature. Then, its cap is sealed completely with non-absorbent cotton and it is covered with aluminum foil and placed in an autoclave for 20 minutes at 121°C and thepsi 15 pressure. Later, we transferred the PDA culture medium in a sterile laminar hood along the flame to glass plates, after cooling the medium, the plates containing the culture medium were placed in incubator at 28°C for 24 hours to detect if the culture medium is contaminated.

2.2. Mycotoxins Production

After 24 hours, the culture mediums which were located inside the incubator and had no contamination were used for cultivating fungus. Then, the laminar hood was disinfected with alcohol cotton and it is covered with aluminum foil and placed in an autoclave. In the end, we removed the Arlene containing soaked rice at room temperature for 2 hour, after the incubation period and the produced aflatoxin and acrotoxin were powdered separately by the mill and after that left it for 20 minutes at 121°C and 15 psi pressure in the autoclave.

2.2.1. Preparation of Essential Materials and Tools for Cultivating Fungus on Rice to Produce Aflatoxin and Acrotoxin

1. Sterile distilled water 500 ml
2. Triton ×100
3. Cotton
4. Sampler
5. 10-ml graduated sterile cylinder

To produce aflatoxin, first we mixed 10 ml of sterilized distilled water with 5μl of Triton ×100, then added it to the plate containing Aflatoxin Aspergillus Flavus NRRL 2999 productive fungus and scratched the surface of the fungus to completely mix the fungus with distilled water. After completing this, we added 500μl of the mixed fungus and distilled water to the autoclave rice that were prepared before. Then, we tightly shut the door of the Arlene and shook it until the solution containing fungus is completely spread over the rice. Next, incubated Arlene containing rice and fungus were kept at 28°C for 21 days. After 72 hours, when fungal myceliums grew on rice, we shook the Arlene and the rice to link them with each other due to the growth of the fungus break. This activity was performed after every 72 hours, 4 times a day. This will provide the needed space to grow fungus on rice. In order to produce acrotoxin from Aspergillus acrasus NRRL 3174, first 10 ml of sterilized distilled water was completely mixed with 5μl of Triton ×100, then the plate containing the aflatoxin Aspergillus acrasus NRRL 3174 productive fungus is added to it. Moreover, the surface of the fungus is scratched completely and the fungus is mixed with distilled water. After completing this, we added 500μl of mixed fungus and distilled water to the autoclaved rice that were already prepared. Then, the door of the Arlene is tightly closed and the solution is shook until the fungus completely spread over the rice. Then, the Arlene containing rice and fungus are incubated at 28°C for 21 days. After 72 hours, when fungal myceliums grow on rice, the Arlene and rice are shook to link each other due to the growth of the fungus break. The procedure is repeated after every 72 hours 4 times a day. This provided the needed space to grow fungus on rice. After the incubation period ended, we placed Arlene containing fungus for 20 minutes at 121°C and 15 psi pressure in the autoclave.

2.2.2. Toxin Maintaining Method

In order to maintain produced aflatoxin and to prevent molding, it should be poured into the metal trays separately after the incubation period and the produced aflatoxin and acrotoxin autoclaved and spread inside the tray completely. Then, we placed trays containing toxin in the aven at 80°C for 24 hours, after that we removed trays containing aflatoxin and acrotoxin from the oven. After that, contaminated rice with aflatoxin and acrotoxin were powdered separately by the mill and each one is inserted into the zipper plastic bags separately and left in the refrigerator (Fig. 1).

Fig. (1). Aflatoxin (A) and Ocrottoxin (B) produced by Aspergillusflavus NRRL2999 and Aspergillusacrasus NRRL 7431, receptively, in temperature of 28°C for 21 d incubation.
2.3. Mycotoxins Assay

The current study to determine AF and OTA concentrations used thin-layer chromatography (TLC) to separate non-volatile mixtures with high performance, the method described by Pons and Goldblatt in 1965 [12] by comparing the fluorescence with the standard. Diffusible zones of AFs and OTA were detected under long wave (365 nm) UV light as blue fluorescent.

2.4. Statistical Analysis

Each sample was analyzed in triplicate using the SPSS 17.0 software and data were calculated for comparison. The results were expressed as the mean ± standard deviation (SD).

3. RESULTS

The data obtained in the study are given in Table 1. The mean levels of AF and OTA estimated in this investigation using PDA as a culture medium and TLC in rice samples were found to be 625 and 478.67 μg/kg of substrate used at 28°C in still culture, receptively. These concentrations were harmonic with the reports of the Iran National Standards Organization about the mycotoxin contamination of cultivated rice.

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Aspergillus Strain</th>
<th>Concentration (μg/g)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>Flavus</td>
<td>B1  531</td>
<td>625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B2  43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1  21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G2  30</td>
<td></td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>Acrasus</td>
<td>Ochratoxin A 478.67</td>
<td>482.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ochratoxin B 3</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>Ochratoxin C 1</td>
<td></td>
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</table>

4. DISCUSSION

In the current study, the author's efforts to design an appropriate method to control the level of mycotoxins in rice consumed in the Persian market to avoid the associated risk of human disorders. Our results revealed that AF and OTA are the predominant mycotoxins detected in rice grains. Moreover, these results alarm us about the potential risks of A. flavus to public health and economic issue. Results close to the results of this study have also been obtained in various previous studies [2, 13, 14]; however, a certain amount of contamination seems unavoidable at present.

A temperature regime of 28°C was ideal for AFs and OTA production. It has been reported that similar results were obtained in some previous studies. Reddy et al. [15] reported that 25°C is the optimum temperature for the production of aflatoxin-B1 by A. flavus isolated from discolored rice grains in the Indian market. The occurrence of mycotoxin contamination is further stimulated by ongoing global warming as reflected in some findings [16]. Furthermore, the Aspergillus genus to boost growth and mycotoxin production requires lower humidity [17]. These results suggest that future studies on the production of mentioned toxins should be conducted at the optimum temperature and humidity.

Due to the variety of structures of mycotoxins, it is impossible to use one standard technique for analysis and/or detection [7]. Screening of samples contaminated with mycotoxins by TLC gets yielded qualitative or semi quantitative results [17]. TLC provides a cheaper alternative to LC-based methods and has an important role, especially in developing countries, for surveillance purposes and control of regulatory limits [18].

PDA as a culture medium allows good growth of Aspergillus strains because these fungus species have exhibited the ability to thrive in standard PDA.

CONCLUSION

Using the methods of PDA media and TLC has shown great potential for mycotoxin analysis and is likely to be popular in the cereals Persian market. A. flavus NRRL 2999 and A. acrapus NRRL 7431 are two important species of AFs and OTA producing, which are considered a source of corruption in rice.

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PDA</td>
<td>Potato Dextrose Agar</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer Chromatograpy</td>
</tr>
<tr>
<td>AFs</td>
<td>Aflatoxins</td>
</tr>
<tr>
<td>OTA</td>
<td>Ochratoxin A</td>
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</tbody>
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CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

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Declared none.

REFERENCES


