AN EFFECT OF THE ROSEMARY (ROSMARINUS OFFICINALIS) EXTRACT ON THE PRODUCTION OF AFLATOXIN B₁ BY ASPERGILLUS FLAVUS

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Abstract: Aflatoxins, metabolites of the fungus Aspergillus flavus, are potent liver toxins and carcinogens in animals, and may also be human carcinogens. There are more than 13 different subtypes of aflatoxin with B₁ being the most toxic. The aim of this work was to investigate influence of the rosemary extract at three different concentrations on the production of the AFB₁ by three isolates of Aspergillus flavus, that showed ability to produce AFB₁ at high concentrations. Samples were analyzed by VICAM’s AflaTest. The rosemary extract at two investigated concentrations (5.00 and 6.50 ml.l⁻¹) decreased content of AFB₁ in YES broth. In the case of isolate 2, the decrease of aflatoxin production was not observed, what means that levels of AFB₁ also depending on the isolate nature. The concentration of 6.50 ml.l⁻¹ showed stronger effect than 5.00 ml.l⁻¹ (isolate 3). The lowest concentration of rosemary extract (3.00 ml.l⁻¹) stimulated production of AFB₁.

Key words: rosemary extract, aflatoxin B₁, Aspergillus flavus

INTRODUCTION

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial effects of natural compounds. Numerous investigations have confirmed the antimicrobial action of essential oils (EOs) in model food systems and in real food (Koutsoumanis et al., 1998; Tsigarida et al., 2000; Rasooli et al., 2008).

Since EOs are considered as generally regarded as safe (GRAS) (Kabara, 1991), the possibility of reinforcing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to attain a balance between sensory acceptability and antimicrobial efficacy. Mycotoxins are secondary metabolites produced by specific filamentous fungi that contaminate agricultural commo-
dities. They are toxic to humans and animals, cause significant reductions in crop yield and cause economic losses (Gourama & Bullerman, 1995). Aflatoxins are highly toxic secondary metabolites produced by the species of Aspergillus, especially A. flavus and A. parasiticus. These fungi can grow on a wide variety of foods and feeds under favourable temperature and humidity. Aflatoxins have been found to contaminate a wide variety of important agricultural products world-wide, e.g., corn, wheat, rice, spices, dried fruits, and nuts. Contamination by aflatoxins can take place at any point along the food chain from the field, harvest, handling, shipment and storage (Giray et al., 2007).

They have been clearly identified as highly toxic, mutagenic, teratogenic and carcinogenic compounds and have been implicated as causative agents in human hepatic and extra hepatic carcinogenesis (Castells et al., 2008). Consumption of mycotoxin-contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes resulting in death (Bathnagar & Garcia, 2001).

Aflatoxin B₁ is the most powerful hepato-cancer agent known in mammals and is classified by the International Agency of Research on Cancer as Group 1 carcinogen (IARC, 1993). Aflatoxin B₁ is the substance without colour, smell or taste. It is stable and resistant to degradation during normal food cooking process. It could be completely destroyed by chromsulfat acid, Na hypochlorite, concentrate NaOH and by being exposed to solar light for an extended period of time (Šutić & Stojanović, 1973).

Presence of aflatoxins is relatively rare in our country (Mašić, 2000). But, we import many foodstuff, including spices, from regions with tropical and subtropical climate that are often contaminated by aflatoxins. Therefore, it is clear that all the examinations of aflatoxins and their producers have great significance, especially if we know their nature.

In addition, many natural compounds found in dietary plants, such as extracts of herbs and fruit extracts, possess antimicrobial activities against Aspergillus species (Soliman & Badeea, 2002; Rasooli & Owlia, 2005). Many spices and herbs, such as cloves, anise and star anise seeds (Hitokoto et al., 1980), basil, cinnamon, marigold and spearmint (Soliman & Badeaa, 2002), garlic and onion (Benkeblia, 2004), thyme (Rasooli & Owlia, 2005), cassia and sweet basil (Atanda et al., 2007) have been reported to inhibit toxigenic and foodborne moulds.

Rosmarinus officinalis L. (family Lamia-ceae), is also known as rosemary. This herb is an evergreen shrub, with aromatic linear leaves. The evergreen shrub originated in the Mediterranean area, but it is today cultivated almost everywhere in the world, primarily for its aromatic leaves. Rosemary is often used widely as a common household spice, and also as a fragrant aromatic flavoring agent in several commercially available products, such as vegetables, prepared meats, baked goods, etc.

Aflatoxin B₁ extracts have a potent antioxidant activity and are widely used in the food industry. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as canosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chain reactions by hydrogen donation (Aruoma et al., 1992; Basaga et al., 1997; Georgantelis et al., 2007). In addition to inhibition of lipid oxidation, several authors have reported that some of the compounds present in rosemary extracts possess antibacterial properties (Del Campo et al., 2000; Djenane et al., 2002).

The aim of this study was to investigate influence of rosemary extracts in different concentrations on the production of the aflatoxin B₁ by Aspergillus flavus.

**MATERIALS AND METHODS**

**Mould**

Three isolates of Aspergillus flavus that showed ability to produce aflatoxin B₁ (220, 18 and 320 µg kg⁻¹) were used. Aflatoxigenic nature of these isolates was confirmed by thin layer chromatography (AOAC, 1995) and VICAM’s AflaTest during the earlier examinations. Isolates were maintained on Sabouraud Maltose Agar...
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(Merck, Germany) at 4 °C. YES broth served as aflatoxin production medium.

Rosemary extract

Rosemary extract prepared in 70 % ethanol (1:2) was obtained from the Institute of Medicinal Plant Research „Dr Josif Pančić” from Belgrade. Working concentrations of rosemary extract were 3.00, 5.00 and 6.50 ml per one litre of YES medium. The influence of all of three concentrations of rosemary extract on three isolates of Aspergillus flavus was examined. Three controls without rosemary extract were also prepared.

Measurement of aflatoxin B₁

Erlenmeyer flasks containing 100 ml of YES broth and R. officinalis extract at appropriate concentrations were inoculated with 5 ml of fungal spores suspension (10⁸ spores/ml). Spore population was counted using a standard Koch method. The flasks were then incubated at 25 ± 2 °C for 12 days in an incubator shaker. Aflatoxin B₁ measurement was determined at the end of the incubation period by VICAM’s AflaTest.

AflaTest from VICAM is the trusted aflatoxin test that produces precise numerical results. Using monoclonal affinity chromatography, AflaTest can isolate aflatoxins B₁, B₂, G₁, and G₂ from feeds, foods and grains at levels as low as 0.1 ppb. Results may be recorded using a digital fluorometer readout with automatic printing devices. AOAC and FGIS established reliability and assurance of the Afla test.

RESULTS AND DISCUSSION

As can be seen from Table 1, addition of rosemary extract at the concentration of 6.50 ml.l⁻¹ decreased the production of AFB₁ by isolate 1 for 31.82% and by isolate 3 for 79.04%, in comparison with the control. In the case of isolate 2, the decrease of aflatoxin production was not observed.

Table 1.
The production of the aflatoxin B₁ (AFB₁) by Aspergillus flavus in YES broth with rosemary extract at the concentration of 6.50 ml.l⁻¹

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of rosemary extract (ml.l⁻¹)</th>
<th>Concentration of AFB₁ (µg.kg⁻¹) YES</th>
<th>Concentration of AFB₁ (µg.kg⁻¹) YES + rosemary extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.50</td>
<td>220.00</td>
<td>150.00</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>18.00</td>
<td>19.00</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>320.00</td>
<td>67.00</td>
</tr>
</tbody>
</table>

Results in tab. 2. show that the addition of rosemary extracts at the concentration of 5.00 ml.l⁻¹ decrease production of AFB₁ by isolate 1 for 31.82% and by isolate 3 for 7.25%, in comparison with the control. In the case of isolate 2, significant decrease of aflatoxin production was not observed.

Table 2.
The production of the aflatoxin B₁ (AFB₁) by Aspergillus flavus in YES broth with rosemary extract at the concentration of 5.00 ml.l⁻¹

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of rosemary extract (ml.l⁻¹)</th>
<th>Concentration of AFB₁ (µg.kg⁻¹) YES</th>
<th>Concentration of AFB₁ (µg.kg⁻¹) YES + rosemary extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.00</td>
<td>220.00</td>
<td>150.00</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>18.00</td>
<td>17.00</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>320.00</td>
<td>300.00</td>
</tr>
</tbody>
</table>

Table 3.
The production of the aflatoxin B₁ (AFB₁) by Aspergillus flavus in YES broth with rosemary extract at the concentration of 3.00 ml.l⁻¹

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of rosemary extract (ml.l⁻¹)</th>
<th>Concentration of AFB₁ (µg.kg⁻¹) YES</th>
<th>Concentration of AFB₁ (µg.kg⁻¹) YES + rosemary extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.00</td>
<td>220.00</td>
<td>1100.00</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>18.00</td>
<td>60.00</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>320.00</td>
<td>2100.00</td>
</tr>
</tbody>
</table>
Results presented in Tab. 3. show that rosemary extract at the concentration of 3.00 ml.l⁻¹ possessed stimulative effect on the production of AFB₁ in all three cases, especially for isolates 3 and 1. The production of AFB₁ by isolate 3 was increased 6.56 times in comparison with the control. In the case of isolate 1 the increase was 5 times.

Investigation performed by Rasooli et al. (2008) showed that aflatoxin biosynthesis of isolate 1 the increase was 5 times.

The antifungal and aflatoxin inhibition efficacy of R. officinalis essential oils may be attributable to the oil compositions (Cardenas-Ortega et al., 2005; Pinto et al., 2006). Antibacterial and antifungal activities of some substances have been reported (Cardenas-Ortega et al., 2005; Pinto et al., 2006).

It has been shown that the activity of rosemary could be attributed to borneol and other phenolics in the terpene fraction (Davidson & Naidu, 2000). The volatile terpenes carvacrol, and p-cymene have been suspected for being responsible for the antimicrobial activity of some essential oils. Also, a group of terpenes (borneol, camphore, 1,8 cineole, -pinene, camphone, verbenonone and bornyl acetate) was reported to possess an antimicrobial activity in rosemary (Davidson & Naidu, 2000).

Antimicrobial activities are mostly attributable to the presence of phenolic compounds such as thymol, and to hydrocarbons like -terpinene and p-cymene with limonene being more active than p-cymene (Vardar-Unlu et al., 2003).

CONCLUSION

Regarding the obtained results of preliminary examinations of the antymycotoxic activities of rosemary extracts, further investigations should be undertaken in order to established a relationship between antymycotoxigenic activity and chemical composition of rosemary extracts. In consideration of different sensativity of three examined isolates of A. flavus on activity of rosemary extract on AFB₁ synthesis, their genetic diversity should be investigated.

ACKNOWLEDGMENTS

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REFERENCES