Biological Degradation of Patulin Mycotoxin Producing from some Fungi in Dried Fruits

1Dr. Shatha Ali Shafiq, 2Dr. Hadi M. Aboud, 1Sami Jabbar Ismael

1Mustansiriyah University - College of Science / Dept. Biology
2Directorate of Agricultural Research - Ministry of Science and Technology

INTRODUCTION

Mycotoxins are natural secondary metabolites produced by microorganisms of kingdom fungi, commonly known as molds that are growing on agricultural commodities and have adverse effects on human, animals and crops, result in health and environmental threat beside economic losses [1]. More than 500 types of mycotoxins have been identified to present, which include the most commonly mycotoxins associated with food and feed that may be concern to consumer food safety: Aflatoxins, Ochratoxin A, Patulin and Trichotheceones [2]. Among the most important mycotoxins was (Patulin)PAT is a β unsaturated lactone and soluble in water and most polar organic solvents – soluble, produced by a number of fungal species belonging to the genera Penicillium, Aspergillus, Byssochlamys and Pacelomyces[3]. The main producer of PAT is the blue mold Penicillium expansum, which is considered as a wound pathogen [4]. It is usually associated with fruits and vegetables especially in apple and its products [5,1]. PAT was first isolated as an antimicrobial active principle
during 1940, from *Penicillium griseofulvum*, during the 1960s, PAT was reclassified as a mycotoxin which was toxic to both plants and animals [6]. Different physical and chemical methods have been established for elimination of fungi producing mycotoxins and contamination of food and feed with toxigenic fungi. Nevertheless, only few of these methods have been accepted for practical uses, a practical and effective method was needed to be developed for the detoxification of PAT contaminated agricultural commodities [7]. Biological method using antagonist microorganisms has received large attention in recent years as a promising alternative, which considers more safety in both human health and ecosystem. The term probiotic was currently referred to ingestive microorganisms associated with beneficial effects to humans and other animals and plants [8,9]. Some strains of lactic acid bacteria group (LAB) and yeast *Saccharomyces cerevisiae* have been reported to be effective in removing PAT from contaminated fruits [10,11].

The aim of the present study included the evaluation the efficiency of Iraqi Probiotic and *Saccharomyces boulardii* as a biological detoxifying agents of PAT production and test their ability to inhibit growth of toxigenic fungal isolates *Penicillium expansum* in liquid culture medium.

**MATERIALS AND METHODS**

Fungal isolates:

Three fungal isolates of *P. expansum* isolated from dried fruits (grape and apricot) collected from different markets in Iraq at Baghdad city and Karbala governorate (from three different samples include (K) Karbala (dried grape), dried apricot of Al-Sadr city (S) and (Z) grape in Al-Utaiyya, were obtained from department of biology - college of Science / Mustansiriya university.

Detection of PAT Toxigenic Fungi:

Glucose-Czapek’s Apple medium (GCA) [12] was used to stimulate PAT toxigenic fungi, conical flasks containing 50 ml of sterilized GCA inoculated with two discs (5 mm in diameter) of 7 days old culture grown on PDA, with three replicates for each treatment, flasks were incubated at temperatures 15°C for three different incubation periods [5,10,15] days in order to select incubation periods for production of PAT. Fungal biomass was separated by filtration through Whatman No.1 filter paper, then the filtrate was re-filtered through a millipore filter (0.45 µm in diameter) to remove fungal spore, 10 ml of filtrate was washed with 20 ml of chloroform in separating funnel, shaken for ten min. the top layer of chloroform was filtrated then reduced at 45°C then remained residue was dissolved in 1ml acetonitrile and kept in a deep freeze until used for PAT detection by (HPLC) the amount of PAT was estimated in comparison with standard PAT through the following formula [13].

\[
\text{PAT conc.} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{Standard concentration}
\]

The percentage of reduction of PAT was calculated using the formula:-

\[
\% \text{ Reduction} = \frac{\text{concentration in tested sample}}{\text{concentration in control treatment}} \times 100
\]

Iraqi Probiotic:

Iraqi probiotic was a Local product obtained from department of plant protection, College of Agriculture – Baghdad University. Each one gram involves numerous microorganisms: *Lactobacillus acidophilus* 10⁸ Colony Forming Unit (CFU), *Bacillus subtilis* 10⁸ (CFU), *Lactobacillus* spp. 10⁸ (CFU) and *Saccharomyces cerevisiae* 10⁸(CFU).

*Saccharomyces boulardii*:

*S. boulardii* was obtained as a powder foreign product from department of Biology / College of Science – Mustansiriyah University. Grown on Sabouraud dextrose broth and then transferred to the solid media.

Suppression of PAT by Biological Methods:

Bio-detoxification treatments using Iraqi probiotic and *S. boulardii* yeast as detoxifying agents:-

1-Suppression of PAT by Iraqi Probiotic:

The method described by (12) to detoxify of PAT was used with some modifications (using of Iraqi probiotic instead of lemon oil) . Iraqi probiotic in two concentrations (0.03 and 0.06) mg/ml was added to 50ml of GCA broth containing toxigenic fungi produce PAT(*P. expansum* isolate from Karbala sample) and incubated at 15°C for 14 days, control treatment involved GCA broth containing toxigenic fungi produce PAT only with no Iraqi probiotic addition, all treatments performed in triplicate then PAT concentration in all treatments were estimated by HPLC and the percentage of Patulin reduction was calculated.
2- Suppression of PAT by *Saccharomyces boulardii*:

*S. boulardii* was used as detoxification bio-agent to detoxify PAT in GCA filtrate. *S. boulardii* was cultivated into Saharab Dextrose Agar (SDA) and incubated at 37°C for 48 hours, two concentrations (10⁸ yeast/ml, 10⁹ yeast/ml) were used by McFarland Standards with 50 ml of GCA broth containing toxigenic fungi produce PAT (*P. expansum* isolate from Karbala sample), then incubated at 15°C temperature at incubation periods 14 days, control treatment involved GCA broth containing toxigenic fungi produce PAT only. All treatments were performed in triplicate and at the end of the incubation periods, PAT concentration in all treatments were estimated by HPLC and the percentage of PAT reduction was calculated.

**RESULTS AND DISCUSSION**

Evaluation of Fungal toxigenic:

Three isolates of *P. expansum* from Karbala, Al- Sadr City and Al- Utaifiyya respectively, were evaluated on GCA medium at 15°C for three incubation periods 5, 10 and 15 days, for their ability to produce PAT. Table (1).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Days</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. expansum</em>K</td>
<td></td>
<td>0.0± 0.00</td>
<td>0.0± 0.00</td>
<td>0.0± 0.00</td>
</tr>
<tr>
<td><em>P. expansum</em>S</td>
<td></td>
<td>11.3±0.01</td>
<td>14.8±0.00</td>
<td>16.5±0.01</td>
</tr>
<tr>
<td><em>P. expansum/Z</em></td>
<td></td>
<td>10.1±0.00</td>
<td>13.9±0.01</td>
<td>13.4±0.00</td>
</tr>
</tbody>
</table>

*Significant difference at p < 0.05

Suppression of PAT by Iraqi Probiotic:

The results of the efficiency of Iraqi probiotic in suppression PAT production in dried grape and apricot fruits was found to be probiotic concentration dependent Table (2).

<table>
<thead>
<tr>
<th>(Conc. Probiotic mg/ml)</th>
<th>Conc. PAT (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>325.0±0.00</td>
</tr>
<tr>
<td>0.03</td>
<td>192.18±0.01*</td>
</tr>
<tr>
<td>0.06</td>
<td>0.0±0.00*</td>
</tr>
</tbody>
</table>

* Significant difference at p < 0.05

The addition of probiotic at 0.03 mg/ml in 50 ml of toxigenic fungal culture medium was significantly reduce the amount of PAT to 192.18 µg/ml with 40.869% reduction ratio, compared with the control treatment 325.01 µg/ml.

On the other hand the effect of Iraqi probiotic at 0.06 mg/ml with 100% reduction ratio in 50 ml of toxigenic fungal culture medium was suppressed production PAT as manifested by disappearance of PAT when examined in HPLC device. This goes back to concentration 0.06 mg/ml with high reduction percentage 100% may be stimulate lactic acid bacteria and the yeast *S. cerevisiae* (probiotics components) to the rapid growth and thus stopped fungus growth that produce the PAT or might have to detoxification of PAT. Likewise Hasan [12], reported the use of 0.2% of lemon oil was more important because it induced 100% inhibition of PAT, at a temperature of 15°C for a period of 14 days and these conditions appropriate for the conditions that have been applied in the experience of smashing PAT by probiotics. The results was compatible with that obtained by Shafiq[14], who recorded the efficiency of Iraqi Probiotic in inhibition of fungal growth *Fusarium graminearum* and Zearalenone production completely in liquid medium using higher concentrations of Probiotic (1.5 and 2 %) w/v. Recently, Karim [15] investigated that Iraqi Probiotic and *Saccharomyces boulardii* had protective effect against mycotoxin Zearalenone ZEN in white mice when they fed a diet contaminated by *F. graminearum* producing Zearalenone for 10 days and ameliorated liver and kidney tissues from Zearalenone toxicity. Numerous studies have pointed to the importance of using the probiotic as bio-agents to remove or destroy the mycotoxins in general [16], whenever there was a diversity of microorganisms and even within the same species in the composition of probiotic, that will lead to exhibit the important role of probiotic in detoxification of mycotoxins.

Suppression of PAT by *Saccharomyces boulardii*:
The results of the activity of two concentrations of *S. boulardii* (10⁸, 10¹⁶ yeast/ml) in suppression of PAT produced by *P. expansum* in toxigenic fungal culture medium, revealed that both concentrations completely detoxification the toxin from the culture medium with Reduction percentage 100%, while it recorded 325.01±µg/ml in treated control Table (3).

<table>
<thead>
<tr>
<th>Concentration of <em>S. boulardii</em>(yeast/ ml)</th>
<th>Conc. PAT (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>325.01±0.01</td>
</tr>
<tr>
<td>10⁸</td>
<td>0.0±0.00*</td>
</tr>
<tr>
<td>10¹⁶</td>
<td>0.0±0.00*</td>
</tr>
</tbody>
</table>

* Significant difference at p < 0.05

The detoxification activity of *S. boulardii* may be due to their ability to absorb PAT on their cell wall. Many studies recorded the ability of yeast cell in the absorb mycotoxins. Ringot and others[17] recorded the adsorption of Ochratoxin A (OTA) onto yeast industry by producing: a vinasse containing yeast cell walls, a purified yeast beta glucan, and a yeast cell wall fraction also noticed that yeast biomass may be regarded as a good adsorbent to reduce of mycotoxins, due to the presence of some specific macromolecules, such as manno-proteins and beta glucans in the cell wall of yeast. Coelho and others [18] found that in the apple juices inoculated with 0.25 g/l of commercial dried *S. cerevisiae* cells (corresponding 1.8 x 10⁷ cells/ml), 96% of PAT was degraded after 6 days of incubation at 25°C under static conditions. Guo and others [19] reported the detoxification of PAT by cell wall of the heat treated *S. cerevisiae* which plays a role in PAT binding and its cell wall integrity especially proteins and polysaccharides are important in the binding process.

REFERENCES


