Abstract

The study was conducted to evaluate the activity of Thuja alcoholic extract to inhibit Aspergillus flavus growth and detoxify the aflatoxin B1 that produced. The results showed high activity of Thuja extract in inhibition of A. flavus growth on culture media that attained to 70 – 25, 85 – 40, 100% with the concentrations 0.2, 0.5, and 1% respectively. The activity of the extract in A. flavus growth inhibition was found associated with high activity in detoxification of afla B1 from contaminated corn seeds. The treatment of contaminated corn seeds with 2% of Thuja extract induced high reduction in afla B concentration. The afla B1 concentration was found to be 752.40 ng/g in treated corn seeds compared with 2620.20 ng/g in control representing 71.35% reduction in the first month reached to 100% in the second month.

Keyword: Aspergillus flavus, Thuja orientalis, Afla B1, detoxification

Introduction

The corn Zea mays L. crop is one of the most important cereal crops in the world that constitute the main part of poultry and animal diet (Abbas and Shier, 2009). It was reported that corn seeds were subjected to infection with many fungi producing mycotoxin in field and during storage including A. flavus that produce aflatoxin B1 (Abbas et al., 1988, Makun et al., 2010).

Mycotoxins are secondary metabolites produced by several fungi on corn seeds in the field and storage. The contamination of corn seeds with mycotoxin is considered as one of the most serious problems confronting human and poultry breeding in many countries in the world (Abbas et al., 2000).

Several strategies were followed to avoid the contamination of corn seeds with the fungi producing mycotoxins and detoxification the mycotoxins from the seeds including chemicals (Coker et al., 1985, Frayssinet and Lafarge 1990, Halima, 2017).

The use of synthetic chemicals and fungicides were found effective in inhibition of A. flavus in storage (Paster et al., 1995).

Due to risks associated with the use of chemicals and the popular trend toward environment friendly compounds in agriculture, the necessity of finding acceptable natural products, effective and safer for human health and environment alternative to chemicals against plant pathogens and mycotoxin contamination is of great interest. It was reported that powder and extracts of many medicinal plant posses activity to inhibit A. flavus growth and aflatoxin production (paranagama et al., 2003, sandosskumar et al, 2007). Other studies reported the potential of plant extract to degrade the aflatoxins (Hajar and Sharma, 2005, Sandosskumar et al., 2007, Sapcate et al., 2005, Halima et al., 2017).

The objective of this study is to evaluate the activity of Thuja alcoholic extract to inhibit Aspergillus flavus growth on culture media and detoxification afla B1 from corn seeds under storage conditions.

MATERIALS AND METHODS

Aflatoxin Production: An isolate of A. flavus producing aflatoxin B1, isolated from corn seeds collected from different sites in Baghdad area and identified by PCR, was used in this study. The fungal isolate was grown on rice seeds, for producing afla B1. Rice seeds 150g were added to 100 ml distilled water in 500 ml flask and autoclaved twice at 121°C and 1.5KG/Cm² for two successive days. The seeds were contaminated with 4 discs, 7mm diameter of A. flavus growth on PDA and homogenized. The flask containing the conta-
minated rice seeds was maintained at 25±2 °C for 21 days oven dried at 50°C and ground. **Afla B1 extraction:** Twenty-Five grams of contaminated rice seeds powder were added to 100 ml of Acetonitrile and water 90: 10 mixtures in a 300ml flask. The mixture was submitted to agitation in flasks shaker for 30 mins and passed through Whatman No.4 filter paper. The filtrate was added to 25ml hexone in separating funnel and gently agitated for 30 sec. The lower layer was mixed with 25 ml distilled water, 8ml of saturated sodium bicarbonate (NaHCO₃), and 25 ml chloroform. The mixture left for 3 min. and the upper layer was mixed with 15ml at NHCl and 20 ml chloroform in separating funnel. The lower layer was passed through filter paper covered with a layer of anhydrous sodium sulfate (Na₂SO₄) and the filtrate was dried by rotary evaporation at 70 °C. The precipitate was dissolved in 1 ml of Acetonitrile : Benzene 2: 98 mixture and conserved under freezing (Balzer et al, 1978).

**Estimation of Afla B1 Concentration:** Afla B1 concentration was determined by high performance liquid chromatography (HPLC) system, model LC 20/0A, Shimadzu Co. Koyata, Japan. Twenty microliters (20 ml) of the final solution were injected in HPLC silica column C18DB (50 x 4.6 mm) 3 mm particle size with mobile phase 0.01N potassium phosphate (KH₂PO₄) solution at pH. 6.0 with flow rate 1.0 ml/min. The absorption values were followed by spectrophotometer at 220nm. and the concentration was estimated by the equation, Afla B1 cons. = Area of sample curve / Area of standard curve × standard solution cons. × dilution factor (Caprita et al., 2007).

**Activity of Thuja alcoholic extract in A. flavus growth inhibition**

**Thuja extract preparation:** Air dried of Thuja foliage and fruits were ground in grinder (willy mill, model No.31). Hundred grams of the powder were homogenized with 200 ml of ethyl alcohol (95%) in a waring blender. The homogenate was agitated in an electric shaker for 24 hrs. and passed through Whatman No.1 filter paper in Buchner tunnel with vacuum. The filtrate was concentrated in rotary evaporator at 50 °C (Harborne, 1973).

**Test for A. flavus growth inhibition:** The concentrated extract was added into sterilized PDA (at 121°C and 1.5 Kg/Cm²) before solidification (50°C) at 0.2,0.5,1% and homogenized. The medium was poured in 9 cm diameter Petri plates and inoculated in the center of 5mm disc of A. flavus growth on PDA (5 days old). The plates were maintained at 25± 2 °C for 7 days and the percentage of fungal growth inhibition was calculated by the equation

\[
\% \text{ of growth inhibition} = \frac{\text{control colony diam} - \text{treatment colony diam}}{\text{control colony diam}} \times 100
\]

The activity of Thuja extract in detoxification afla B1 in contaminated corn seeds: Twenty ml at 2% Thuja alcoholic extract were added into 500ml flask containing 125g of contaminated corn seeds with 2626-2 ng/g afla B1, from contaminated rice seed powder as determined by HPLC. Twenty ml of water were added into control flasks. The flasks were maintained at room temperature and the concentrations of AlfaB1 were followed by HPLC for 3 months.

**RESULTS AND DISCUSSION**

**Source of corn seed contamination:** Corn seeds collected from different sites in Baghdad area were found to be internally infected by A. flavus under storage conditions. This infection may be started in the field (Preharvest) and continue to develop under poor storage conditions.

It was reported that growth of Aspergillus spp. from surface sterilized maize grains was evidence in poor grain storage (Christensen and Kaufmann, 1974). The infection in the field may start from maize residues in the soil containing fungal conidia. It has been found that maize residues in the cultivated field were colonized by Aspergillus spp. (Shearer et al., 1992). The residues became more important if they colonized by insects vectoring Aspergillus to ripening maize ear (Lussenhop and Wicklow, 1990).

The infection of maize grains by A. flavus requires entering breaks in the pericarp. These breaks may be formed by insects, birds, or climatic stress during ripening of ears. It was reported that corn earworm was implicated as providing entrance into the ear (Zuber and Lillehag, 1993). Smart et al.,
(1990) showed that A. flavus enter the seeds through microscopic breaks in the tesla.

**Activity of Thuja extract in A. flavus growth inhibition and Afla B1 detoxification:** The addition of Thuja alcoholic extract into culture media (PDA) at 0.2, 0.5, 1% induced high reduction in A. flavus growth with inhibition percentages 70.25, 45.40, 100% for the three concentration respectively shown in table 1.

Table 1: Inhibition activity of Thuja alcoholic extract against *Aspergillus flavus* growth in culture media

<table>
<thead>
<tr>
<th>% extract cons.</th>
<th>Inhibition Percentage</th>
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<tbody>
<tr>
<td>0.2</td>
<td>70-25</td>
</tr>
<tr>
<td>0.5</td>
<td>85-40</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

The activity of Thuja extract against A. flavus growth may come from its contents of active compounds mainly tannic acid and certain of these compounds penetrate fungal cell and react with cell membrane leading to modify its permeability and provoke cell death. It was reported that many medicinal plant extracts including Borage and French jasmine showed high inhibition of several pathogenic fungi growth (Murth et al., 2009).

The activity of Thuja extract against A. flavus growth was found associated with high activity in detoxifying afla B1 from corn seeds. The treatment of corn seeds, contaminated with 2626.w ng/g of afla B1, with 2% Thuja alcoholic extract induced high reduction in afla B1 concentration, that attained to 752.40 ng/g at reduction percentage 71.35% in the first month, reached to 100% in the second month and results are presented in table 2. The reduction in aflaB1 concentration may result from that active compounds in the extract react with active group in the mycotoxin causing the opening of its lactone ring and degradation or may converted to non-toxic compounds.

Extracts of many medicinal plants have been reported to inhibit A. flavus growth and aflatoxin detoxification (Parangama et al., 2003 Hajar and Sharama, 2005, Sandosskuman et al., 2007).

Table 2: Activity of Thuja alcoholic extract in detoxification aflatoxin B1 in corn seeds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aflatoxin B1 Concentration ng/g</th>
<th>1st month</th>
<th>%reduction</th>
<th>2nd month</th>
<th>%reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn seeds contaminated with aflaB1 control</td>
<td>2626.20</td>
<td>————</td>
<td>2435.0</td>
<td>————</td>
<td></td>
</tr>
<tr>
<td>Corn seeds contaminated with aflaB + 2% Thuja extract</td>
<td>752.40</td>
<td>71.35</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Non-Contaminated Seeds</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION**

The use of Thuja alcoholic extract showed high activity in A. flavus growth inhibition on culture media and afla B1 detoxification in corn seeds. These results indicated that natural products may be promising as a source of safer and more effective and provide an alternative way to avoid the contamination of food and feed with fungi and aflatoxins.

**REFERENCES**


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