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DETOXIFICATION OF PATULIN MYCOTOXIN USING BY SEAWEED EXTRACT OF BROWN ALGAE ASCOPHYLLUM NODOSUM

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ABSTRACT
The current study was focused on the ability of the foreign product seaweed extract of brown algae Ascophyllum nodosum to detoxifying of Patulin mycotoxin producing from Penicillium expansum. The results showed that seaweed extract of brown algae at concentrations (1.5 and 3) % v/v inhibited the vegetative growth of Penicillium expansum the inhibition percentage reached to 79% and 90 % at the concentrations (1.5,3) % v/v respectively in culture medium Glucose-Czapek`s Apple broth (GCA). However the results of test the ability of two concentrations of seaweed extract of brown algae in Patulin detoxification from broth culture of (GCA) for 14 days of incubation showed completely detoxification of Patulin compared with control treatment 325.01 µg/ml.

KEYWORDS: Patulin – Detoxification - seaweed extract.

INTRODUCTION
Mycotoxins are natural secondary metabolites produced by filamentous fungi of kingdom fungi , 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 Trichothesences.[1] 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, Byssochlomys and Pacelomyces.[2] The main producer of PAT is the blue mold Penicillium expansum, which is considered as a wound pathogen.[3] It is usually associated with fruits and vegetables especially in apple and its products.[4,5] PAT was first isolated as an antimicrobial active principle during 1940_s from Penicillium griseofulvum, during the 1960_s, PAT was reclassified as a mycotoxin which was toxic to both plants and animals.[6]

Most countries have set up maximum admissible levels of PAT in processed products from apples such as apple juice, apple concentrate juice, which have been identified 50 µg/L as recommended by the Food and Drug Administration.[7] PAT exhibits sever toxicoses to human and animals exposed to the toxin via consumption of contaminated products, it has been shown to have toxic effects and can harm the immune system and gastrointestinal tract. Also shows carcinogenic, teratogenic, and mutagenic properties toward different mammalian cells including human cells.[8,9,10,11]

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.[12]

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.[13,14] Some strains of lactic acid bacteria group (LAB) and yeast Saccharomyces cerevisiae have been reported to be effective in removing PAT from contaminated fruits.[15,16] The aim of present study included inhibition of growth of toxigenic fungus P.expansum and detoxification of Patulin mycotoxin using by Seaweed extract of brown algae Ascophyllum nodosum in vitro.

MATERIAL AND METHODS
Fungal isolates
Three fungal isolates of P. expansum isolated from dried apricot fruit collected from different markets in Iraq at

Baghdad city included Palastine street (P), Al-Sadr city (S) and (Z) in Al- Utaifiyya, were obtained from department of biology - college of Science / Mustansiriyah university.

Detection of PAT Toxigenic Fungi

Glucose-Czapek`s Apple medium (GCA)[17] 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, flasks were incubated at temperatures 15℃ for 14 days in order to select incubation periods for production of PAT. Fungal biomass was separated by filtration through Whatmman 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℃ 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.[18]

PAT conc. = Peak area of sample Peak area of standard × Standard concentration

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

% Reduction = conc. in control treatment - $concentration\ in\ tested\ sample$$concentration\ in\ control\ treatment$×100.

Seaweed Extract

Seaweed extract of brown algae Ascophyllum nodosum is obtained as a commercial foreign product from College of Agriculture -Baghdad university.

Suppression of PAT by Seaweed Extract

The method described by Shafiq and others[19] to detoxifying of PAT Seaweed extract of brown algae Ascophyllum nodosum in two concentrations (1.5 and 3) % v/v were added to 50ml of GCA broth containing toxigenic fungi produce PAT (P. expansum isolated from Palastine street P) and incubated at 15℃ for 14 days, control treatment involved GCA broth containing toxigenic fungi produce PAT only with no seaweed extract addition, all treatments performed in triplicate then PAT concentration in all treatments were estimated by HPLC and the percentage of Patulin reduction was calculated.

RESULTS AND DISCUSSION

Evaluation of Fungal toxigenic

Three isolates of P. expansum from Palastine street, Al-Sadr City and Al- Utaifiyya, were evaluated on GCA medium at 15$^{\circ}C$ for 14 days, for their ability to produce PAT**.** (Tabe.1)

Table 1: The quantitative estimation of PAT in dried apricot fruit samples collected from various local markets in Baghdad.

Fungal isolates	Con. of PAT. μg/ml
P. expansum/P	325.01
P. expansum/S	51.1
P. expansum/Z	79

In this respect the amount of PAT production by fungal tested isolates in this study agreed with McCallum and others[20] who found substantial differences among P. expansum strains in terms of growth kinetics and PAT production in three different apple ciders. The results also compatible with[17,19] who showed maximum production of PAT using same medium (GCA) and different isolates of P. expansum and they noted that GCA was found to be the best medium supporting maximum PAT production by P. expansum isolates.

Effect of Seaweed Extract on Growth of P. expansum

The anti-fungal activity of seaweed extract are represented by the percentage of inhibition between two concentrations (1.5,3) % v/v of seaweed extract were increased with the increasing of concentration, the percentage of inhibition was reached to 79% and 90% at the concentrations (1.5,3) % v/v respectively in culture medium GCA compared with control treatment.

This result agreed with the findings of many studies[21,22,23] they screened the most active compounds in algae, biochemical analysis are being undertaken to determine the structure and nature of compounds responsible of the bioactivity of the extracts with high antibacterial activity. Not only the presence of a particular compound which makes these organisms, interesting but also their huge diversity and the possibility of not only harvesting them but also of growing them at different conditions, leading to an enrichment of some bioactive compounds.

Suppression of PAT by Seaweed extract

The results of the activity of two concentrations of Seaweed extract (1.5 and 3) % v/v 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 325.01μg/ml in control treatment Table -2.

Table 2: The Efficiency of Seaweed extract in reduction of PAT in GCA broth.

Concentration of Seaweed extract % v/v	Conc. PAT (μg/ml)	Reduction %
0.0	325.01	0.0
1.5	0.0	100
3	0.0	100

The detoxification activity of Seaweed extract may be due to their bioactive compounds and their interaction

with the patulin biosynthetic pathway. Our finding agreed with Nezha and others[24] how reported the ability of ethanolic extracts marine algae (Cystoseira tamaricifolia) at the concentration of 5% to inhibit Mycotoxins formation in Aspergillus flavus and also Shafiq[25] was shown the ability of sea weed extract of brown algae Ascophyllum nodosum as a detoxifying agent against mycotoxin Zearalenone production.

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