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عزل وتشخيص الاسبرجلس فليفس بالطرق الجزيئية من الحليب في محافظة ديالى

رسالة مقدمة إلى مجلس كلية الطب البيطري – جامعة ديالى كجزء من متطلبات نيل درجة الماجستير

في

الاحياء المجهرية البيطرية

من قبل

ميس جبار خميس

بكالوريوس طب وجراحة بيطرية

بإشراف

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1.Introduction

Since ancient times, milk and its derivatives have been one of the most popular foods. This value put the food hygienists in a real challenge to provide safe milk to consumers with maintaining its nutritional value. The consumption of milk was estimated to be every day for a lot of people as it was the source of many nutrient essential for human (Langat,2017).

Milk contamination by fungal through environment, tools, milk handlers and packaging materials. Molds and yeasts are identified as an essential reason of spoilage of various dairy products (Khalifa *et al*., 2013; Pal and Jadhav, 2013; Pal *et al.,* 2014). Same fungi are identified by producing mycotoxins that are harmful to human health, these fungi are *Aspergillus*, *Fusarium* and *Penicillium* (Sengun *et al.,* 2008).

Mycotoxins have a multiplicity of adverse impact on both humans and animals when ingested (Hampikyan *et al*., 2010). *Aspergillus flavus* and *Aspergillus Parasiticus* were the most filamentous fungi producing aflatoxin that affect in human and animals feed staff (Baskaya *et al.,* 2006). Other aflatoxin producing species included *Aspergillus nomius* , *A. pseudotamarii, A. bombycis, A. toxicarius, A. parvisclerotigenus, A. ochraceoroseus* , *A. rambellii* and *E. venezuelensis* (Ito *et al.*,2001; Frisvad *et al.,* 2004; Frisvad *et al.,* 2005; Reiter *et al.,* 2009).

Tropical and subtropical environment offered the essential requirements for the growth of Aspergillus species as they had the ability to grow on different media. As they grew, they produced aflatoxins that found their way to contaminate agricultural products especially when they were stored under imperfect conditions (Prandini *et al*., 2009; Kensler *et al*., 2010).

More than 20 kinds of aflatoxins were produced by *Aspergillus parasiticus* and *Aspergillus flavus* but the four main types are aflatoxin B1, B2, G1and G 2 (Inan *et al.,* 2007). Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) were identified as milk toxin, they are produced from hydroxylated of AFB1, this was followed by the metabolization action of cytochrome P450 associated enzymes in liver (Giray *et al,*2007). Major species, that have ability to produce aflatoxin are *Aspergillus flavus* and *A. parasiticus* (Yu *et al.,* 2004). The most toxic toxin reported was Aflatoxin B1 and known as potentially hepato-carcinogenic (Bennett and Klich , 2003). Aflatoxin M1 is excreted through milk, feces and urine of animals following utilization of the AFB1 contaminated feeds (Aycicek *et al*., 2005; Fallah *et al*., 2009).

The metabolite of Aflatoxin B1 was AFM1, it was reported to be present human and animals' milk when they were feed on grains that contained molds with AFB1. There was sufficient evidence that AFM1 less toxic than AFB1, but both toxins had potential hazardous on human health. Cell transformation, DNA damage, chromosomal anomalies and gene mutation were reported to be due to the effect of both above-mentioned toxins (Klich , 2002).

The metabolite of AFM1 from AFB1 was detected in the milk of human and animals after 12-24 hours from the first ingestion of moldy grain contaminated with AFB1, its concentration decreased to invisible levels within 72 hours when the consumption of such milk was avoided (Aliabad *et al.,* 2012).

Many researches have proved that the maximum permitted level for AFM1 in raw milk and heat-treated milk at (0.05 parts per billion)0.05µg /L (Hussain and Anwar, 2008; Dashti *et al*., 2009; Amer and Ibrahim, 2010 ; Kamkar *et al.,* 2011; Tsakiris *et al.*, 2013).

There are different diagnostic methods used for the detection of the Aflatoxin in food materials including thin molecular techniques, Enzyme Linked Immunosorbent Assay (ELISA), thin layer chromatography (TLC), immunochemical techniques, immune-sensors, and High-performance liquid chromatography (HPLC).

Aims of the study :

1- Isolate and identify *Aspergillus flavus* from crude milk samples of different species and from of different locations of Diyala Province by routine methods.

2-Extract aflatoxin from isolated fungi.

3-Detect aflatoxin in crude milk samples.

4- Apply molecular identification of aflatoxigenic *A. flavus* and phylogenetic analysis of detected strain.

5-Estimate the sensitivity of *A. flavus* isolated strain to antifungal drugs.