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# Isolation and Identification of Fungal Species in Kurdish Walnut Morphologically and using some Molecular Technique.

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## ABSTRACT

Walnuts are world widely consumed in many local dishes in southeast Asian countries, especially in Iraq-Kurdistan-Tawella which is one of the major walnut cultivars area in this region, particularly in Hawraman region. Three species of filamentous fungi *Asperagillus* were identified in this study including *A. niger, A. flavus, A. parasiticus* and in walnut product including (salted walnut). Therefore, *Aspergillus* species, which produced a significant amount of aflatoxin, ochratoxin and T2- toxin contamination in walnuts during storage are becoming major concerns due to the insufficient condition that favors the growth of aflatoxigenic and ochratoxigenic fungi. The current study thus aimed to morphologically and molecularly identify and characterize the *A. niger, A. Flavi, A. parasiticus* isolated from Kurdish walnuts and its product. The DNA isolation from all fungal species wee r from agar cultures after inoculating and purifying for more times by using commercial kit (Bio basic INC kit). Agarose gel electrophoresis fractionation of the fungi DNA, showed clear individual bands. *A. niger* distinct band is 500 bp, *A. flavus and A. parasiticus* distinct *band is 1032* bp. ELISA technique was applied for detection quantitatively for aflatoxin, ochratoxin and T2 toxin in Kurdish walnut. As stated in table two the result of ELISA method used for quantitative detection of aflatoxin, ochratoxin and T2 toxin is 5.9, 10.4 and 129 part per billion ppb respectively, it is safety range for all toxins.

***Keywords:*** *Asperagillus niger, A. flavus, A. parasiticus.* morphological, Aflatoxin, ochratoxin molecular tools, ELISA. PCR techniques.

## INTRODUCTION

Walnut (*Juglans regia* L), is a significant nuts utilized around the world, and known to advance the development of various microorganisms including toxigenic and pathogenic contagious species. It has been much of the time announced that tree nuts produce mesmerizingly toxigenic forms, for example, *Aspergillus flavus, A. parasiticus*, as different examinations are expressed that Asperagillus species have capacity to develop in nourishment item, for example, nuts including nut, pecans were found to convey such creatures. (Bayman *et al.,* 2002) announced that the most widely recognized growths in nuts were Aspergillus, Rhizopus, and Penicillium. *A. niger, A. avus, A. parasiticus*, and *A. nomius* were expressed from reviews directed in the US, Europe, Australia, and South America. (Jorgensen *et al.,* 2005) showed that nearness of different Aspergilli and Penicillia including, *A. niger, A. avus, Penicillium crustosum*, and so forth is of unique tension since it has been discovered that a few individuals from these species are equipped for creating aflatoxin ochratoxin and T-2 poisons. Aspergillus segment Flavi (*A. niger, A. parasiticus, and A. flavus*) are known to deliver the harmful and cancer-causing mixes aflatoxins (AFs). Normally happening mycotoxins, for example, AFs, OTA, have likewise been educated from tree nuts. In the event that the dampness of the dried items is kept at the best possible levels, they ought not help form developing. At the point when their water movement (aw), excessed, 0.65, they become inclined to rot by some postharvest parasitic pathogens including Aspergilli and Penicillia genera relying upon the aw level. (Pitt *et al.,* 2009). The nearness of such living beings may diminish the nature of the item being disturbed and cause genuine medical issue for customers. In spite of the fact that there are gives an account of parasitic professional les of tree nuts and dried natural products exist in the writing, the proceeding with changes in fungicide utilized may have activated variety in mycobiota on them after some time. Tournas,2004 secluded some parasitic species including *A. niger, A. ochracious, A. carbonarious* in rasins, and, while (Hocking, 2006) announced the nearness of Aspergillus segment Nigri and *A. ochracious* was disengaged from grapes item like raisins nearness of OTA in dried organic products, for example, currants, raisins, and sultanas has likewise been accounted for in the writing. (Jorgensen *et al.,* 2005; Pitt *et al.,* 2009), exhibited that The dampness has incredible job in dried item and ought to be kept in tolerant level. they ought not bolster form development. At the point when their water movement (aw), be that as it may, outperforms 0.65, they become inclined to waste by some postharvest parasitic pathogens including xerophilic aspergilli and erotica relying upon the aw mark. (Pitt, 2009). nearness of such living beings could impact on item quality and wellbeing of items and be the reason for genuine wellbeing worries to shoppers' nourishment supplies, including, nuts and species with OTA has been

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recorded generally. Mycotoxins are delegated the major nourishing danger factor, higher than engineered contaminants, plant poisons, nourishment added substances, or pesticide deposits (Kuiper-Goodman, 1998; Bennet and Klich, 2003). nourishment tainting by mycotoxins has been unsurprising as a general wellbeing risk (JECFA, 2002). Aflatoxins B1and Ochratoxin A just as aflatoxin M1 and the Deoxynivalenol (DON) have been arranged in the four criticalness Mycotoxins of nourishment and feed contaminants.

**Morphological distinguishing proof of parasitic species in pecan**

Morphological distinguishing proof is regularly utilized for ID of parasitic species and it depends on likeness and dis-similitude or contrasts of morphological characters. Beginning ordered work on Fungal spp was relying upon morphological attributes including both essential qualities of full scale conidia, small scale conidia and chlamydeous, and optional attributes including development rate and pigmentation are delivered in development phases of the contagious cell (Nelson *et al.*, 1983; Leslie and Summerell, 2006). Though, state of full scale conidia is a significant component to separate species. The states of large scale conidia can be little, crescent, ordinary, fusiform or needle like and are assessed by their size, septa and the state of the apical and basal cells. Apical cell shape can be gruff, papillae, snared or decreasing, while basal cell can be foot-molded, stretched foot-formed or particularly prolonged (Leslie and Summerell, 2006).

**Chemical Linked Immune Sorbent Assay (ELISA)**

Chemical Linked Immune Sorbent Assay (ELISA), is a counter acting agent based measure that is normally used to decide of Mycotoxins. ELISA gives fast screening, by utilizing numerous units monetarily accessible for measurement of every single significant Mycotoxin including AFTs, ZEA, DON, OTA, fumonisin, and T-2 poison (Holtzaple *et al.*, 1996; Magliulo *et al.*, 2005). ELISA techniques have been affirmed in a wide assortment of nourishment and feed networks (Pereira *et al.*, 2014). The standard of ELISA depends on the serious cooperation between mycotoxins (going about as an antigen and doled out antibodies related to poison compound conjugate for some, coupling destinations (Pittet, 2005; Turner, 2009). There are a few examinations have been utilized this procedure for assurance of Aflatoxin B1 in ground nut, corn, and wheat (Pal *et al.*, 2004). The measure of counter acting agent bound poison catalyst conjugate will control the level advancement of shading that is mean changing in shading relying upon measure of immune response which bound with poison – protein conjugate (Yao and Hruska, 2014). One kind of ELISA technique is Immunochemical strategies which decide numerous subsidiaries of a mycotoxins; depending of the cross-reactivity of the counter acting agent.

**Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) is one of exact and delicate technique for recognition of microorganism species just as it is utilized for discovery of contagious spp. and their poisons (aflatoxigenic and ochratoxigenic species) (Bhatnagar *et al.*, 2006). For the most part, the exactness of the PCR was seen as 70-85%, where PCR confirmed to be a fast symptomatic method for recognition of parasitic genome straightforwardly from controlled examples (Bagyalakshmi *et al.*, 2007; Hassan *et al.*, 2013). The particular and delicate of PCR test the is helpful for the recognizable proof of contagious contaminations (Bialek *et al.*, 2002; AbdEl-Yazeedet *et al.*, 2008 and 2011). DNA is the primary basic and significance transporter of PCR technique recognizable proof, which is the hereditary material of every microorganism (Moretti, *et al.*, 2017). There are a few troubles portrayal steps of DNA extraction, initial one is can be impacted by certain inhibitors during extraction (McCartney *et al.*, 2003), second issue and urgent one is modest quantities of DNA gathered during extraction ventures from a set number of contagious cells, the most third troublesome issue in the confinement of parasitic DNA is to break contagious cell divider without causing harm of DNA genome which is just mean harm of parasitic cell divider just (Bir et al., 1995). In PCR strategy for specifically intensify DNA groupings that done by 30-40 tedious cycles groundwork toughening and enzymatic augmentation and age a large number of duplicates of the DNA arrangements preliminaries are 18-25 bases long planned specifically to tie of forward and other to corresponding successions to target DNA section (Meyer *et al.*,1999). PCR-based strategies have created numerous pieces of study in light of the fact that the enzymatic ramifications of DNA can be cultivated in vitro from limited quantities of material. PCR-based strategies have likewise been joined with different procedures, for example, RFLP or settled PCR to identification genotype of various microorganisms (Gasser, 2006).

## MATERIALS AND METHODS

All fungal species were isolated from Kurdish walnuts that have been gathered from a field in Tawella region which is has a place with (has a place with Halabja governorate, Kurdistan area of Iraq) during October 2016. The Walnuts item were protected in plastic ziplok holder in fridge until research center use. The contagious societies were moved to cups contained potato dextrose juices (75ml) for single conidial confinement. The conidial suspensions of the way of life were acquired by spore suspension strategy, at that point hatched for 7 days. A solitary province was moved onto new Potato Dextrose agar plates after purging for more than multiple times got unadulterated state of each, the parasitic disengages were moved to sanitized plates for cleaning and distinguishing proof. The developed organisms were assessed with 40 amplifications on a slide, recolored with lacto phenol to distinguish parasitic structures, and recognized based on their province morphology and spore attributes. Species recognizable proof was set up by utilizing Ultra Clean Microbial DNA disengagement unit (Bio fundamental - INC). adhering to the producer's directions.

PCR intensification of detaches was performed utilizing a Standard Thermo cycler (Bio RAD (Bio-essential from). 25 µl response ace blend that contained 5× PCR cushion, 1 µM of groundwork, Corporation, 2012) and 20 ng of DNA layout. A lot of groundworks was utilized; (Arif et al., 2011) (Li and Hartman 2003). to intensify 700 bp part. The PCR cycling was led as follows: beginning DNA extraction 94°C for 85 s, trailed by 35 patterns of denaturation at 95°C for 35 s, tempering at 57°C for 57 s, augmentation at 72°C for 90 s, last expansion at 72°C for 10 min.

The gels were recolored with ethidium bromide (EtBr) and the DNA groups were envisioned under Ultra

violet (UV) light. The DOC print framework utilized for catching pictures. Target DNA section band with estimated 690 bp (Nitschke et al., 2009) was extracted from the gel, trailed by the decontamination of PCR items utilizing following the maker's suggestions. Bio essential INC EZ-10 Spin Column Fungal Genomic DNA Mini-Preps Kit FT82012 Version 4.1 ISO9001 Certified.

Three arrangements of groundwork were utilized right now recognition of Asperagillus species and two sets for aflatoxigenic and ochratoxegenic organisms were utilized as following.

**Isolation and Identification of *Asperagillus* species**

inoculating 25 g of each (Kernel, whole walnut (kernel +shell) in sterilized flask contain 75 ml of PDB (Potato dextrose broth) and put in shaker incubator for 48 hours at 28 $^0$C and 150 rpm for growth of fungal species after growth of each species of fungal cell from pure samples and inoculate it on PDA and incubated for 5 days and also inoculating on three types of media were (MEA malt extract agar, SDA, Sabaroud dextrose agar, CMA Core meal agar and SNA agar) as showed in figure 4-1.

Purified isolates were identified based on their characterization according to identification kye of *(*leslie and Brett;2006; Mounjouenpou *et al.,* 2012; Vega *et al.,* 2012, Nurbaya and Tutik *et al.,* 2014). Pure culture strains were incubated in Potato Dextrose Agar (PDA) medium for 7 days and 25°C Leslie and Summerell, (2006).

Inoculum size of each *Fungal* species was adjusted to be 1.0 × $10^4$ conidia per ml by hemcytometer then serial dilutions were made ($10^{-1}$ - $10^{-3}$). with100 µL of sterile peptone water in1.5 mL was spotted on each both sides of hemcytometer then covered with cover slides. Average number of conidia/ml was counted using the following formula:

**Average of conidia = Numbers of cell/Number of squares**

**Number of conidia= Average of conidia x dilution factor x $10^4$**

**Determination of Mycotoxin by enzyme-linked immunosorbent assay ELISA technique**

Determination of Fungal toxin (Aflatoxin, Ochratoxin, T-2 toxin) in Kurdish Walnut (Kernel, Shell +kernel) determined by kits provided by (Neogen) company, using enzyme-linked immunosorbent assay (ELISA) in the laboratory of Veterinary hospital in Sulaimanyah. Samples of walnuts (whole and Kernel) were numbered, randomized, and blind-coded. Toxins extraction were obtained by homogenizing grinded Walnuts (10g for OTA detection, 5g in case of T-2 and AFs detection) with 100 ml of Ethanol (%70 OTA detection, %50 for T-2 and AFs detection) The 100 µg/mL stock solution was used to spike walnut-negative samples. Extractions were performed following the manufacturer's instructions. ELISA was rigorously performed according to the manufacturer's instructions (Veratox kits; Neogen). After blending the material was filtered with Whatman filter paper number 1.00 the filtrate was used for additional analysis. Commercially available immunoassay kit Veratox for quantitative analysis of aflatoxin and ochratoxin test-(Neogen Crop, Lansing, MI) was used. the usage kit was based on competitive direct enzyme linked to amino sorbent assay kit. The antibodies captured the analyze and conjugated to the enzyme (horse reddish peroxidase). Tetra methyl benzidine /hydrogen peroxide was used as a substrate for color development. In the final step should add stopping solution to stop the reaction. The color intensity was inversely sample for 5 min at room temperature, then centrifuged at 9000 rpm for another 5mint, and filtrated with Whatman filter paper. Each filtrate was applied to ELISA analysis. Optical densities of the developed colors were measured in comparison to known concentrations of our reference standards by using ELISA reader at 630 nm.The ELISA reader can measure the concentration of Mycotoxin. All reagents were necessary provided in the kit. Concentration of mycotoxins was calculated by Log/log it Software Awareness Technology Inc. (Anonymous, 2000; Stoloff *et al*., 1991).

**Table 1. Primers used in this study for detection of mycotoxigenic fungi AFs and OCA**

| Fungal species | Type of toxin | Sequence | Expect size | Annealing temp | Reference |
|---|---|---|---|---|---|
| *A.parasiticus* | Aflatoxin | For-TATCTCCCCCCGGGCATCTCCCGG<br>Re CCGTCAGACAGCCACTGGACACGG | 1032 bp | 55 ºC | Sachova,2003 |
| *A.niger* | Ochratoxin | For- ATGGGTAAGGAGGACAAGAC Rev-GGAAGTACCAGTGATCATGTT | 650 bp | 55 bp | Donnel, 1998<br>Sugita,2004 |

**Gel electrophoresis**

Agarose gel DNA electrophoresis was practiced by the changed strategy for Saghai-Maroof *et al.,* (1984). Two grams of agarose (BioRad agarose, Qiagen) was set up in 98 ml 1 TAE (Tris/Acetate/EDTA) support to give a 2% arrangement and warmed to breaking point in a small scale wave. The arrangement was reasonable to cool to 60ºC preceding the expansion of 2 ml ethidium bromide (Et Br) (10 mg/l in water to a last centralization of 0.5 mm/ml) and completely blended. The gel was poured in glass plates. Polymerase Chain Reaction items PCR (as expressed in Iheanacho *et al.,* 2014) was gradually over-burden (2 ml) cautiously into the wells. A voltage source was applied is 5V/cm was to the gel for the electrophoresis run and PCR item (Fig. 2). Vacutec Gel documentation framework was utilized and item size fixed by difference to the Middle Range Fast Ruler. The atomic size of DNA procured after extraction was dictated by gel electrophoresis. The gel electrophoresis admissible for the detachment and perception of DNA sections from *A. niger, A. parasiticus* and *A. flavus*.

## RESULTS AND DISCUSSION

After justification to $10^4$ cells for each samples by spore suspension method which were incubated in 75 ml of Potato dextrose broth in shaker incubator for two days at 28 $^O$C at 120 rpm and inoculating on four types of medium are (Potato dextrose agar PDA, Malt extract agar MEA, Sabaroud dextrose agar SDA, Core meal agar CMA. This may be due to their occurrence incidences, especially *A. flavus* as compared to *A. parasiticus* in walnut, and their individual capacities to produce aflatoxin and ochratoxin However, their identifications based on identification keys were high related to that obtained in this study.

Our results were in fine agreement with those found by Adebajo and Diyaolu (2003), who isolated *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Syncephalastrum*, the

most predominant isolates were: *A. niger*, *A. restrictus*, *A. flavus*, Rostami *et al*. (2009) who isolated fungal genera from nuts.

Detection and quantitative determination of Mycotoxin by ELISA method for quantitative determination of aflatoxin, ochratoxin and T2 toxin in Kurdish walnut this results are agreed with many studies including this result highly garment with studies of (Slotwinski *et al.*, 2018) and this result is involving standard range (safety ranges) in food and feeds (nuts as well as walnut).

The colonies of these species of fungi isolated from walnut (shell+Kernel) samples, Overall data indicated that 69.5, 65.2 and 52.1% of walnut samples were originate to be contaminated with *A. flavus*. *A. niger* and *A. parasiticus*, respectively. Hence, and Mycotoxin producing as illustrated in (Table 2).

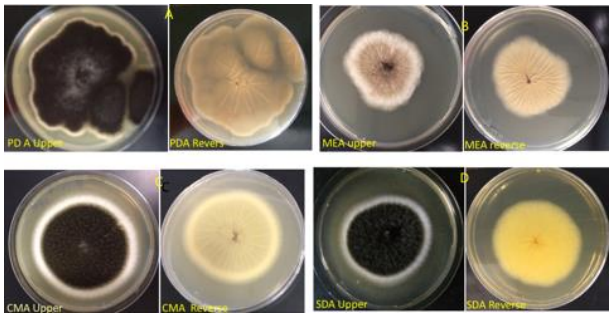



**Fig. 1. Upper and reverse of *A. niger* on four types of medium (PDA, SDA, CM, MEA incubated in 28$^{O}$C for 7 days (Botton et al., 1990; Samson et al., 2007).( Pollastro et.,2006)**

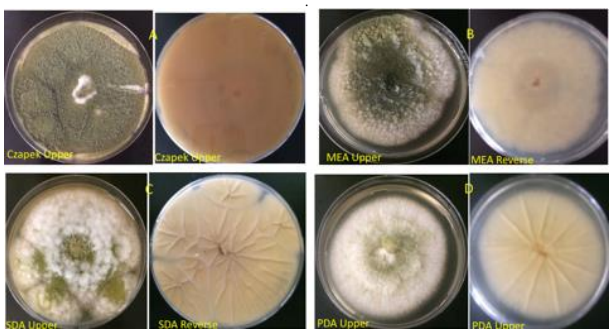



**Fig.2. Upper and reverse of *A. parasiticus* which inoculate *on* four types of medium (PDA, SDA, CMA and MEA) incubated in 28$^{O}$C for 7 days (Botton *et al.*, 1990; Samson *et al.*, 2007).**

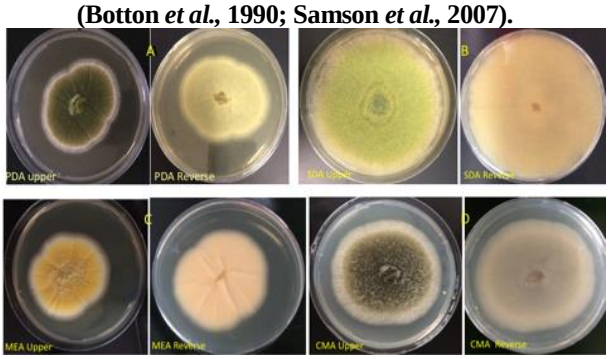
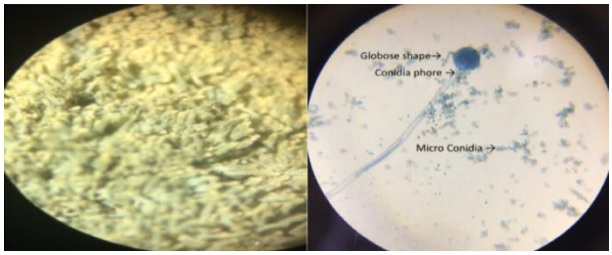
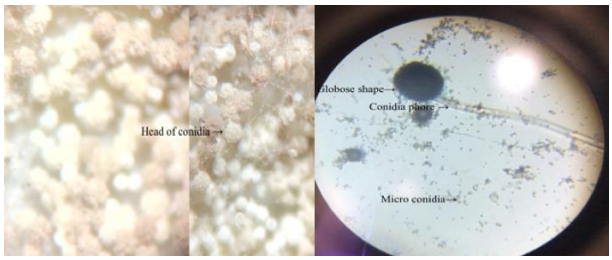


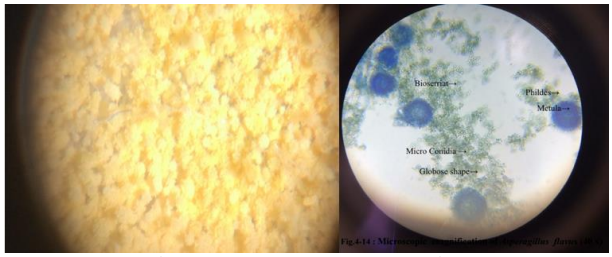

**Fig. 3. Upper and reverse of *A. flavus* which inoculating on four types of medium (PDA, SDA, CMA, MEA) incubated in 28$^{O}$C for 7 days our result is well agreed with (Frisvad *et al.* 2005; Lee *et al.* 2004).**




***A. parasiticus*** ***A. parasiticus conidia***




***A. niger conidia*** ***A. niger***

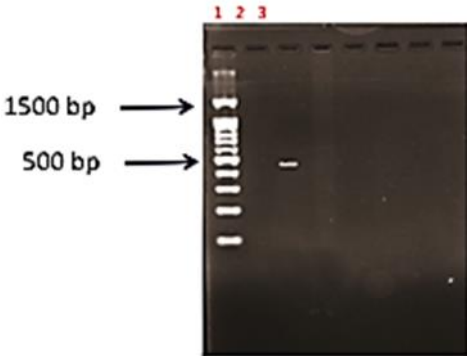



***A. flavus conidia*** ***A. flavus***

**Fig. 4. Microscopic characteristic of Aspergillus species (*A. niger* ,*A. flavus and A.parasiticus* ) 10 and 40 X magnification. as shown each conidia, micro conidia, head of conidia of each species. (Pak et al., 2007).**

**Table 2. Detection of aflatoxin, ochratoxin and T2 toxin by ELISA technique**

| Name of Toxin | Concentration (ppb) | Standard (ppb) |
|---|---|---|
| Aflatoxin | 5.9 | 10 |
| Ochratoxin | 10.4 | 10.8 |
| T2 Toxin | 129 | 150 |




PCR–based detection of *A. niger* DNA product, reaction was carried out in volume of 25 µl (10-100 ng) of template DNA, 1 µl of each primer, and 10 µl of master mix PCR product were detected on 1% agarose gel in TAE 1X buffer gel electrophoresis analysis using of primer (pcr product containing DNA (Nurbaya et al 2014). (Lane 1 Ladder 100-1500 bp, lane 2 NO DNA for sample Lane 3 sample content of *A. niger* 500 bp). (Samson *et al*., 2007). using specific primers as described in previous section.

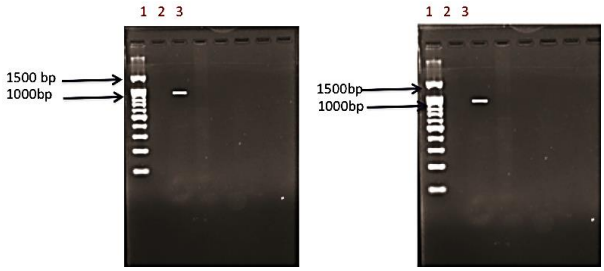



**Fig.5.** **1** **2**

**(1) Lane 1 - DNA ladder 100-1500 bp Lani 2-Negative control (No DNA), Lane 3-*Aspergillus flavus* DNA template with size band 1032 bp). (2) *A. parasiticus* Lani 1-DNA ladder Lani 2 Negative control Lani 3 DNA template of *A. parasiticus* molecular weight band sizes: 1032 bp, Geiser *et al.*, 2000; Vaamonde *et al*, 2003; Pildain *et al*., 2008; Hussein and Juber (2015). Using specific primers for detection of mycotoxigenic species.**

he incidence and contamination levels of *A. flavus and A. niger and A. parasiticus* in the Kurdish walnuts were calculated, and data reviewed in **Table .2** Isolates of pure fungal strains for DNA extraction were subculture on fungal isolate extract saccharose (YES) broth medium and incubated for 7 days at 25º C according to modified method of Polymerase chain reaction in the method of Fredlund *et al.*, (2008). Extraction of DNA was performed using a DNA extraction Mini kit (Bio basic INC) according to the manufacturer's (Bio basic kit) modified protocol. The purified DNA was stored at 20 ºC until further analysis. PCR reaction to amplify the *afl D* gene of aflatoxin-producing fungi Individual reactions had 2.5 ml of DNA sample solution mixed with 5 ml master mix which content (Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at best concentrations for effective insinuation of DNA templates by PCR), 1 ml of each primers and 13.5 ml nuclease free water to kind up a result volume of 25 ml. The PCR was performed in Eppendorf tubes placed in 36-well rack of the. The following temperature range of 95ºC for 20s, 65º C for 20s and72ºC for 30s were used for the 35 cycles PCR. Which agrees with other studies from (Mohankumar *et al.*, 2010; Godet and Munaut, 2010).

**Discussion**

Walnut it has absolutely and conceders important diet in our daily meal in Asian country especially in Iraq, it plays an importance rolls in daily meals due to highly rich in proteins, antioxidant, fat, some vitamins and minerals content that a person needs a day. walnut is a rich source in terms of omega 3 which is useful for a regular health and vital rich source of many importance materials. In this study, a reasonable ELISA method was standardized, legalized and its performance was compared with the other molecular tools technique method of organized steps for detection and quantification of mycotoxins the aflatoxin and ochratoxin in Walnut samples is the unique and accurate used regularly by the laboratory for analysis of a aflatoxins in foods and feed (Sinha *et al.*, 1999). Polymerase chain reaction (PCR) is one of accurate method for detection of microorganism species as well as it is used for detection of fungal and their toxins (aflatoxigenic and ochratoxigenic species) (Bhatnagar *et al*., 2006). Generally, the accuracy of the PCR was found to be 70-85%, where PCR confirmed to be a rapid diagnostic technique for detection of fungal genome directly from controlled samples (Bagyalakshmi *et al.*, 2007; Hassan *et al.*, 2013). The specific and sensitive of PCR assay the is useful for the identification of fungal infections (Bialek *et al.*, 2002; AbdEl-Yazeedet *et al.*, 2008 and 2011).

For enzyme linked-immunosorbent assay (ELISA) in is accurate and simply detecting mold in food and nuts or agricultural products, there are various studied about ELISA systems for detecting the mold spoilage and contaminated in food and agricultural products including nuts and its products (Yong ,1995). For ELISA is affords rapid and correct screening. There are several kits commercially offered for detection and quantification of all main mycotoxins including AFs, AFM1, OTA, DON, T-2 toxin, ZEA, fumonisin, and Although HPLC and ELISA techniques have been broken for a period of times (Sinha ,1999).

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**عزل وتشخيص بعض الخلايا الفطرية الموجودة في الجوز الكوردي (*Juglans regia* L) وذلك على الاساس الشكل الظاهري واستخدام بعض الطرق الجزيئية.**

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اجريت هذه الدراسة في قسم البايولوجي – كلية العلم – جامعة السليمانية بهدف التحري عن التلوث الفطري في الجوز الكردي في منطقة الهورامان (ته ويلة) و ان الجوز الكردي له مميزات الخاصة و النادرة و له اهمية الكبيرة في الوجبات الغذائية و خاصة الصباحية بانه يحتوي على العناصر الغذائية و المهمة و الفيتامينات و المعادن و يعتبر المادة المهمة باحتوائه على المضادات الاكسدة و انواع المعادن النادرة و عند الكشف عن اللوث الفطرى اظهرت النتائج بأن الاجناس الفطرية (*Aspergillus niger, Aspergillus Flavus, Aspergillus Parasiticus*) ونسبة الظهور كالاتي: 52.1و 69و 65% على التوالي. وأن هذة الاجناس لها قابلية على انتاج السموم الافلا والاوكرا بعد تنميتها على الوسط الغذائي (PDA) وفحص بطريقة الجزيئية (التفاعلات البلمرة التضاعفية) والتفاعل الانزيمي المرتبط ايضافة الى فحص على الاساس الشكل الظاهري على الاطباق الجزء العلوي والسفلي كما مبين في الشكل (1و2و3) وان هذة العزلات لها القابلية على انتاج الاوكراتوكسين والعزلة الثانية والثالثة لها قابلية على انتاج افلاتوكسين ولكن ضمن النسب القياسية حسب مواصفات القياسية العالمية والاوروبية. وان نتيجة الطريقة الجزيئية (التفاعلات البلمرة التضاعفية) اظهرت النتائج ظهور الحزم بالحجم المطلوب.