The Republic of Iraq
Ministry of Higher
Education and Scientific Research
University of Babylon \College of Science
Department of Biology

Identification of Ochratoxigenic Fungi and Characterizations of Ochratoxin A in some Dried Fruits of the Local Markets in Babylon Province

A/Thesis
Submitted to the Council of College of Science at University of Babylon in Partial Fulfillment of the Requirements for the Master Degree in Biology

By
Abeer Hadi Naji Mohsen

B.Sc. / Biology (2012)

Supervised by

Prof. Dr. Ibtihal Muiz Abdul Mahdi Al Hussaini
Prof.Dr. Jawad K. Abood Al_Janabi

University of Babylon \College of Science

2021 A.D **1442 A.H**

بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا ﴾

صدق الله العلي العظيم

سورة النساء : الآية(113)

Supervisors Certification

We certify that this thesis entitled " Identification of Ochratoxigenic fungi and Characterizations of Ochratoxin A in some dried fruits of the local markets in Babylon province " has been prepared under my supervision at the Department of Biology, College of Science, University of Babylon, in partial fulfillment of the requirement for the Degree of Master Science, By the student " Abeer hadi naji Mohsen abbas

Signature:
Name: Dr. Prof. Dr. Ibtihal Muiz Abdul Mahdi Al Hussaini
Scientific degree: Professor
Address: College of Science- University of Babylon
."**Date**: / /2021

Signature:
Name: Dr. Jawad kadim Abood Al-janabi
Scientific degree: professor
Address: College of Science- University of Babylon
Date: / /2021

In view of the available recommendations, I forward this thesis for debate by the examining committee

:Signature

Name: Dr. Adi Jassim Abd Al-Razzak

Title: Assistant Professor

Address: College of Sciences / University of Babylon

Date: / /2021

Supervisors Certification

We certify that this thesis entitled " Identification of Ochratoxigenic fungi and Characterizations of Ochratoxin A in some dried fruits of the local markets in Babylon province " has been prepared under my supervision at the Department of Biology, College of Science, University of Babylon, in partial fulfillment of the requirement for the Degree of Master Science, By the student " Abeer hadi naji Mohsen abbas

Signature:
Name: Dr. Prof. Dr. Ibtihal Muiz Abdul Mahdi Al Hussaini
Scientific degree: Professor
Address: College of Science- University of Babylon
."**Date**: / /2021

Signature:
Name: Dr. Jawad kadim Abood Al-janabi
Scientific degree: professor
Address: College of Science- University of Babylon
Date: / /2021

In view of the available recommendations, I forward this thesis for debate by the examining committee

:Signature

Name: Dr. Adi Jassim Abd Al-Razzak

Title: Assistant Professor

Address: College of Sciences / University of Babylon

Date: / /2021

Acknowledgments

The first thing to begin with is praising Allah and foremost, prayer and peace be upon the best for His Prophet Mohammed and his household.

My special thanks with respect and appreciation go to my supervisor Dr. Jawad K. Abood and Dr. Ibtihal Muiz for her helpful guides, advice and encouragement throughout this study.

I would like to thank the Deanery of college of science, University of Babylon, for providing the necessary facilities and thanks go to a head of Biology Department .

I am extremely grateful to Dr. Hussien AL-Dahmoshi, for providing all the essential needed for the completion of the present work.

My deepest thanks also to Al-Hillah Teaching Hospital Staff . Also , I would like to thank the staff of the College of Science, University of Babylon for their kind assistance.

Abeer2021

Dedication

I dedicate to all the martyrs,

especially my dear father

To the wellspring of love

My dear mother

., altruism and generosity

To all my brother and sisters

To the people closest to myself

my faithful husband

.To my soul, the coolness of my eyes, and my heartbeat

my children

To all whom I received advice from

I present to you the summary of my scientific efforts

Summary

Dried fruits of (*Vitis vinifera* L. , *ficus Carica* L. , *prunus armeniaca* L. , *Juglans regia L*) are considered "healthy foods" and are used in biscuits, cakes, and other baked foods. This study aimed to characterize Ochratoxin A (OTA), and using beneficial fungi to control the ochratoxin producing fungi. Experiments include the presence of toxigenic fungi and OTA in these dried fruits, Morphological characterizations of contaminant fungi, their occurrence, and frequency, molecular identification of *A. ochraceus*, the capabilities of this fungus to produce ochratoxin A through Thin Layer Chromatography (TLC) and HPLC techniques in addition to the possibilities of ochratoxigenic fungi control using beneficial fungi: In total, 380 fungal isolates were obtained from Iraq's most popular dried fruits, such as, *Vitis vinifera* L , *Ficus Carica* L., *prunus armeniaca* L., *Juglans regia L,* and common plums. Using morphological cultural characteristics such as colony color, margins, texture, and colony reverse colors, several different species, belonging to four different genera, were identified: *A. niger (80), A. terrus (50), A. flavus (55), A. ochraceus (40). Fusarium (66) Nigrosora (35) and Penicillium (54)*. The percentage of contaminating fungi that appeared on dried fruits varied depending on the type of dried fruits and the contaminant fungus. Apricots, Raisins, and Iraqi Raisins all had rather a uniform infection to the dried fruits. While infection in other dried fruits (*Vitis vinifera* L , *Ficus Carica* L., *prunus armeniaca* L., *Juglans regia L*, and common plums) was variable. studies have shown that the toxin-producing fungi, especially *Aspergillus*, are among the main pollutants in dried fruits and that the fungi *A. niger and A. flavus* are the most frequently isolated species in dried fruits.

Apricots (35%) had the highest level of contamination by contaminant fungi, followed by raisins (32%), Iraqi raisins (26.2%), Common plum (2.6%), figs (2.4%), and hazelnuts (1.6 %). The results of TLC showed that 6 isolates of *A. ochraceus* were mostly produced ochratoxin in different types of the studied dried fruits. The results confirmed the verification of TLC result through the PCR product analysis of 18S rRNA gene ITS2 gene from extracted DNA of *A. ochraceus* isolates showed that positive *A. ochraceus* isolates at (420bp).

The results showed considerable antagonistic abilities of *Trichoderma harzianum* and *Pleurotus ostreatus* to inhibit *A. niger* and *A. ochraceus* using three methods: direct antagonism through dial culture and volatile and non-volatile substances. Both bioagents had a significant inhibitory effect on the radial growth of both contaminant fungi, but the inhibitory caused by *T. harzianum* was greater by using culture filtrate. Otherwise, *P. ostreatus* was a greater inhibitory effect on the growth of both pathogenic that *T. harzianum.*

Despite differences in inhibitory impact between *T. harzianum* and *P. ostreatus* against Ochratoxin contaminated fungus *A. niger* and *A. ochraceus*, both showed successful antagonistic activity and the ability to compete against them.

List of contents

List of Tables

List of Figures

Abbreviation	Complete Terms
ITS	The internal transcribed spacer
PCR	Polymer chain reaction
PDA	Potato dextrose agar
SDA	Sabouraud dextrose agar
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
ANOVA	A one way analysis of variance
OTA	Ochratoxin A
UV	Ultra violet
PK	Protein kinase
PCR	Polymer chain reaction
PGI	percentag inhibition rowthg
SE	Standard error
LSD	Least significant difference

1- Introduction

Fungi are the main spoiling and toxin contaminating agents of food and feed during storage and processing making them unsuitable for human and animal utilization and causing big economic loss. Improper storage conditions offer a beneficial environment for *Aspergillus* spp. growth and mycotoxin(s) production (Milicevic *et al* ., 2010).

Mycotoxin contamination of foods is a significant food safety problem for grains and other agricultural products (Eaton and Groopman, 1994; Payne, 1998). Mycotoxins are secondary fungal metabolites which are toxic to humans and animals because they can cause serious illnesses (Essono *et al*., 2007). Aflatoxins, ochratoxin A, zearalenone, fumonisins, ergot alkaloids, enniatins, patulin, Alternaria toxins, and trichotheceneses have been identified as essential mycotoxins (Barac, 2018).

Ochratoxin was detected in South Africa in 1965, representing a community of Linked compounds manufactured by *Aspergillus ochraceus*, *Penicillium verrucosum* and other species of *Penicillium* (Bennett *et al*., 2003).

In this group, ochratoxin A (OTA) is the most important fungal toxin than ochratoxins B and C. Potent nephrotoxicity, hepatotoxicity, cytotoxicity, and immunotoxicity are caused by OTA. It is also a possible teratogenic and immunosuppressive drug. It is potentially a human carcinogen and is of particular concern because it can accumulate in animal meat (Walker *et al*., 2002; Battilani *et al.*,2003).

In general, the most common fungus belonging to the genus *Aspergillus* and *Penicillium* since these forms are characterized by growth in different environmental ranges with a high enzymatic capacity that enables them to remain over than in any fungus (Pitt, 1994).

More than 250 known species belong to the genus *Aspergillus*. Most of them, such as antimicrobial agents, organic acids such as citric and gluconic acids, and enzymes such as lipases and amylases, are industrially essential for their secondary metabolite development. Some of them are highly toxic, called mycotoxins, which cause various health problems, especially cancer (Gregory, 1973; Abarca *et.al.*, 2004)

Dried fruits, including date and related products, are largely consumed and, because of their sweet taste and nutritional values, are growing in producing and importing countries. Since dried fruit is normally eaten directly without further processing, it is very essential to be mindful of the safety and quality of these items through the determination of mold load and AF contamination (Amezqueta *et al* .,2012).

In Iraq, quite limited studies relating to Ochratoxin A was achieved at Duhok Province reported that dried vines fruit were highly contaminated with a broad spectrum of filamentous fungi, they found that 60% of juice samples were contaminated with ochratoxin A, its levels ranging between 0.37- 1.85 ng/ml as detected by L C-M S /M S technique. Other information is not available yet about the natural occurrence of OTA in foodstuffs in Iraq. , as Saadullah and Abdullah (2018),

These fungi can contaminate crops prior to harvesting and more frequently during storage and thus contribute to the contamination of dried grapes, grape juices and various types of wine (Welke 2019). The objective of this study is to determine the occurrence and characterization of ochratoxigenic fungi and ochratoxin A in some dried foods commonly consumed in Iraq.

The main steps:

1. Morphological and microscopic study of the contaminated fungi fruits in different markets in Babylon Province.
2. Study the capacity of the isolated fungi to produce Ochratoxin A using High-performance liquid chromatography (HPLC).
3. Potential study for the inhibition of growth and ochratoxigenic fungi by *P. ostreatus* or *T. harzianum*.
4. Molecular characterization of Ochratoxin A in fruits-contaminated fungi.

2 Literature Review

2-1 Mycotoxins

Mycotoxins are a group of fungal secondary metabolites that contaminate certain agricultural products at various stages, including pre-harvest, post-harvest, storage and food processing chain, resulting in poor crop quality, yield loss, impaired animal health, and high mortality rates, in addition to posing a health risk to humans. It can cause acute or chronic severe toxic effects in humans and animals when consumed.

Mycotoxins are primarily formed by molds from the genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium*, which can develop on a variety of foods and feeds. The first three of these genera are the key contributors to spoilage and mycotoxins production in fruits and during storage. These fungi are the most common fruit spoilage molds, and they cause significant losses in the food industry. There are over 100,000 species of fungi that produce over 300 metabolites that are harmful to humans and animals. Human organs such as the liver, kidneys, nervous system, muscular system, digestive system, genital system, and respiratory system are all affected. AFs, Patulin (PAT), Alternaria toxins, and ochratoxin A (OTA) are the most common mycotoxins associated with fruits (Efuntoye, 1996; onková *et al*., 2006).

Mycotoxin contamination can occur during the development of the crop (pre-harvest) as well as after harvest (post-harvest). Preventive steps, such as the careful use of insecticides and fungicides, irrigation to avoid humidity stress, harvesting at maturity, and improvement by genetic resistance to fungal attack, are all included in good agronomic practices during the pre-harvest season. Controlling the humidity and temperature of the stored commodity during the post-harvest period will have a

significant impact on the degree of fungal activity and, as a result, mycotoxin production (Bryden, 2007).

The existence of mycotoxins is directly linked to certain aspects of food and feed storage, environmental, and ecological conditions. Despite the presence of numerous toxigenic fungi, no mycotoxin was found in any of the tested samples (whole kernel, nut, or ground meal*). Penicillium* was the most common isolated genus in each of the three forms, followed by *Alternaria and Cladosporium* (Xu et.al., 2011).

2-2-1 Mycotoxin production from isolated fungi

The presence of mycotoxins in foods is becoming a growing source of concern. Specific fungi species create these substances. Patulin (Scott *et al*. 1977) and ochratoxin A (Majerus and Otteneder 1996; Zimmerli and Dick 1996) are mycotoxins found in grapes and grape products.

The fruit-rotting fungus *P expansum* produces patulin, while *Penicillium verrucosum* and *Aspergillus ochraceus* produce ochratoxin A. Apple and grape juices have also been found to contain patulin (Scott *et al*. 1977; Moss 1998). In the United Kingdom, there is a suggested cap for patulin in apple juice of 50lgl1. *Penicillium* expansum also makes citrinin, although it wasn't contained in apple or grape juices (Scott *et al*. 1977). Isolates of the common species *Aspergillus niger* have been known to contain ochratoxin A on rare occasions (Abarca and Bragula ,1994).

The closely related *A. carbonarius*, on the other hand, is a much more popular producer (Varga *et al.*,1996). (Heenan and Shaw 1998), and a much more significant source of ochratoxin A. These species are common in tropical foods (Pitt ,Hoking *et al*.,1993) and can withstand sun drying

(Pitt,Hoking ,Miscamble et al., 1998*). A. carbonarius* is a major source of ochratoxin A, which can be found in dried grape fruits, wines, and possibly coffee.

2-2-2 Ochratoxins

Some Aspergillus species (principally A. *ochraceus and A. carbonarius*) and possibly *A. niger* industrial strains and some Penicillium species, especially *P. verrucosum,* produce ochratoxins. The most common and important fungal toxin in this community is ochratoxin A, while Ochratoxins B and C are less important.

2-2-3 Ochratoxin A

A.ochraceus (Steyn *et al*., 1965) , a species with natural habitats in drying or decaying vegetation, seeds, nuts and fruits. *A. Ochraceus* and closely related species are widely distributed in dried foods of various kinds (Pitt *and Hocking* 1997)

Ochratoxin A is a poisonous substance that can kill animals (Krogh and Hald *et al*.,1974). Since Ochratoxin A is fat soluble and difficult to excrete, it accumulates in contaminated animals' depot fat, where it is then ingested by humans who consume pork. Bread made from barley or wheat may also contain the toxin.

Ochratoxin A was also present in human blood throughout most of Europe, with concentrations as high as 35 (J.g/kg), according to Castegnaro Plestina R *et al,*1991), and in human milk at similar amounts (Bretholtz-Emanuelsson *et al.*,1993). While no strong evidence of human disease has been found, such levels suggest that ochratoxin A is a widespread problem in Europe.

Isolates of the common species of Aspergillus may produce Ochratoxin A on rare occasions (Abarca and Bragulat, 1994) The closely related *A. Carbonarius*, on the other hand, is a much more popular producer (Varga *et al*.1996) and a much more critical source of Ochratoxin A (Varga *et al*.1996) (Heenan *et al* ,1998). These species can survive sun drying and are common in tropical foods (Pitt and Hocking *et al*., 1993; Pitt and Hocking AD, Miscamble BF *et al*., 1998). In dried vine fruits, and possibly coffee, *A. Carbonarius* is a major source of Ochratoxin A.

2-2-4 Production of OTA

Ochratoxin A is a mycotoxin discovered in maize-based products infected with *Aspergillus Ochraceus* by (van der *et al*. 1965). OTA is chemically known as N-{[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl]-carbonyl}-3-phenyl-L-alanine. Four years later, OTA was isolated by van Walbeek *et al*,.1969, from the culture of *Penicillium verrucosum.* In the 1960s, OTA was identified as one of the first classes of animal-toxic fungal metabolites, which, along with the AFs, launched the distinct and diverse science of mycotoxicology. OTA production is currently dominated by two types of fungi. *Aspergillus alliaceus, Aspergillus ostianus, Aspergillus sclerotiorum, Aspergillus sulphureus, Aspergillus melleus, Aspergillus petrakii, Aspergillus glaucus, Aspergillus niger, Aspergillus foetidus, Aspergillus carbonarius, Aspergillus albertens* (Varga et al.,1996).

from the culture of *Penicillium verrucosum*. OTA was described as one of the first groups of fungal metabolites that are toxic to animals, which, with the AFs, launched the distinctive and diverse science of mycotoxicology in the 1960s. Nowadays, two groups of fungi are mainly involved in OTA production. In tropical regions *A. Ochraceus* is probably

the main source, though several other aspergilli are also able to produce OTA including strains of *Aspergillus alliaceus, A. ostianus, A. sclerotiorum, A. sulphureus, A. melleus, A. petrakii, A. glaucus, A. niger, A. awamori, A. foetidus, A. carbonarius, A. albertensis, A. auricomus and A. wentii* (Varga et al.,1996).

2-3÷ Physico-Chemicals Properties of Ochratoxin A

OTA is a weak organic acid with a pKa value of 7.1(Keeper and Scott,1989 Bredenkamp,1989) and a molar mass of 403.8 g.mol-1. This molecule has a colorless to white crystalline structure and emits a bright green fluorescence under UV light in acid medium and a blue fluorescence in alkaline medium (El KhouryAtoui, 2010).

OTA is soluble in polar organic solvents (alcohols, ketones, chloroform) at acid and neutral pH, slightly soluble in water, insoluble in petroleum ethers, and insoluble in saturated hydrocarbons. This molecule is soluble in aqueous sodium bicarbonate solution and all alkaline solutions in general when in alkaline conditions.

When crystallized from benzene as a solvate, it has a melting point of about 90 °C. Xylene, on the other hand, has developed non-solvated crystals with a melting point of 169 °C. The high stability of OTA is one of its distinguishing features. It has been demonstrated that it can withstand acidity and high temperatures. Thus, after foodstuffs have been infected, fully removing this molecule is extremely difficult (El Khoury, Atoui, 2010) showed that the OTA is only partially degraded under normal cooking conditions. Furthermore, this molecule can withstand three hours of 121°C high-pressure steam sterilization, and even at 250°C, it is not completely destroyed (Boudra *et al*.,1995).

2-4-Ochratoxin A in dried fruit

Ochratoxin A is primarily present in cereals and cereal-based products. This group of commodities has been identified as the primary source of ochratoxin. In exposure tests conducted by the European Commission, a single exposure accounted for 50% of overall dietary Ochratoxin A exposure in humans European Countries (European ,1997; Commission, 2002). Apart from cereals and cereal products, Ochratoxin A can also be present in a variety of other foods, such as coffee, cocoa, wine, spices, dried fruits, grape juice and meat products from non-ruminant animals fed infected feedstuffs. Since protozoa in the stomachs hydrolyze Ochratoxin A into non-toxic metabolites before absorption into the blood, ruminant animals such as cows and sheep are usually immune to its effects (Kiessling *et al*.,1984)

Because of their high sugar content and other nutrients, dried fruits are susceptible to fungal and mycotoxin contamination. Mold infection in dried fruits can occur during the ripening stages of the fruit, after it has fallen from the tree, and during the drying process (Ozayi *et.al*., 1995).

The most common fungi isolated from dried fruit samples of black sultanas, plums, figs, , and white sultanas belonged to the genus Aspergillus, with *A. niger* being the most common, followed by *A. Ochraceus*, *A. flavus*, and *A. Carbonarius*, respectively. Although, there was no fungal infection in the apricot samples. This finding may be explained by the fact that these fruits were treated with sulfur dioxide to prevent darkening and fungal growth (Pitt and Hocking, 1997).

2-4-1: Fig (*Ficus Carica* L.)

There are two types of figs on the market: fresh and refined. While the refined market refers to whole dried figs sold in retail consumer packages (for direct consumption) or in bulk for processing, the fresh market refers to whole dried figs sold in retail consumer packages (for direct consumption). The bulk industrial materials are sold to bakeries and food manufacturing companies (primarily cookie and energy bar companies), which use them to make value-added products like cookies, pastries, sauces, and jams.

After the figs have matured and ripened on the tree, they can be dried in one of two ways: tray-dried or sun-dried. Traydried figs are harvested when they reach tree-ripe maturity (Crisosto *et al*., 2010), the white cultivars are treated with SO2, and all of the figs are spread out on trays to dry in the light. Since these figs are treated with SO2 when they are young, they have a larger surface area to absorb it. Sun-dried figs, on the other hand, are completely ripened on the tree before being abscised and dropped to the ground. They're SO2 is applied to white cultivars during postharvest storage. The desired final moisture content of dried figs

Approximately 17% of the material (Crisosto *et al.*,2011). Without the use of a mold inhibitor, the maximum moisture content permitted at retail is 24 percent, and 30 percent with the use of a mold inhibitor (such as, potassium sorbate). Sun-dried figs are sold in the United States, while tray-dried figs are sold in Asian markets as a specialty item (Matt Jura's personal communication). Dried fruit of white cultivars are often treated with SO2 during postharvest storage to prevent enzymatic and nonenzymatic browning caused by the Maillard reaction (Karadeniz et al., 2000).

Since the purple black skin conceals any darkening, "Black Mission," the only dark cultivar used for dried fig processing, does not receive SO2. Color preservation is a critical commercial factor, as shown by a sensory evaluation of dried figs and fig paste that found color to be the most significant factor in deciding acceptance level (Sen *et al*., 2010). Meanwhile, all dried cultivars are preserved with potassium sorbate (Perera, 2007).

Due to the high moisture content of dried figs, which continues to rise after drying and during storage, this antimycotic agent is needed. Between 200 and 600 ppm of potassium sorbate has an antifungal effect, whereas levels greater than 600 ppm can be detected by the taster (Nury and Bolin ,1962).

Dried figs can be infected with xerophilic organisms like *Aspergillus*, which can lead to postharvest spoilage due to mycotoxins. While *A. niger* and related species are common, infection by members of the *Aspergillus* section *Flavi* has been linked to more severe effects, most notably the development of AFs, which is depicted as a major issue in some countries (Embaby *et.al*., 2012; Farjood and Banihashemi, 2013).

In two cultivars of Californian Figs, Doster and Michailides (2007) found a high incidence of *A. niger, A. alternative*, and *Ulocladium atrum*. Simmons (2007), on the other hand, believes that Alternaria diseases in figs are caused by *A. fici and A. ficini. A. alternata, Botrytis cinerea, Cladosporium, Penicillium, and Rhizopus* species are among the other fig diseases recorded (Snowdon, 1990).

2-4-2 Raisins (*Vitis Vinifera* L.)

The fruits are dried using solar energy or hot air. The most common method for raisin production is to use sunlight. Raisins have been sun-dried from grapes since 1490 BCE (Mencarelli *et al*., 2005).

Grapes are currently handpicked from the plant, placed on clean trays between vine rows, and dried in the sun for two or three weeks. are low in fat and cholesterol and high in fiber, potassium, iron, calcium, and vitamin B. As a source of energy, they only use natural sugars. These are high in antioxidants, in addition to sugars, basic amino acids, and fatty acids. 4,5 points Because of their freshness, attractive green color, and sweet flavor, green raisins are usually sold for two to three times the price of sun-dried raisins made from the same fresh grapes. Red, black, and yellow raisins are sun dried on rooftops and on the ground6, while green raisins are dried in the shade in ventilated homes. The main issue with raisin production by field drying and storage is the production of mycotoxins caused by fungi. To avoid fungi formation, warm temperatures (20–30°C) and high humidity must be avoided, and good ventilation through the raisins is needed. To prevent the development of fungi and the subsequent production of mycotoxin, solar dryers are used to minimize drying time and regulate drying conditions. Raisins can be made by passing hot air over grapes in a perforated rotary drum dryer. This method produces raisins faster, but it takes more energy and careful temperature control to prevent quality loss. Since the waxy cuticle of the grapes influences the rate of moisture diffusion through the berries, pretreatments are used to speed up the drying process. Pretreatments include dipping in hot water or using acid, caustic, and ethyl or methyl oleate emulsions. The pretreatments speed up the drying process while also improving the consistency. By altering the waxy layer structure at

the grape surface, surface treatment speeds up the drying process and lowers the internal resistance to water diffusion.

Raisins are further processed to produce raisin juice and raisin paste. Raisin juice is made by repeatedly leaching raisins with water, resulting in a pure extract of raisins (Mencarelli *et al*., 2005). In a vacuum pan, the collected liquid is evaporated to create a self-preserving concentrate with a minimum of 70% natural fruit soluble solids. Raisin juice is used in a wide range of foods, including dairy, confectionery, and bakery. Raisin paste is made entirely of raisins, which are extruded through a fine mesh film. Raisin paste may be used to add color and taste to a dish.

2-4-3: Apricot (*prunus armeniaca* L.)

is a member of the Rosaceae family. Rosaceae is one of the largest angiosperm families, with around 3,400 species spread throughout northern temperate regions of the globe, including almonds, peaches, apples, plums, cherries, and berries. Apricot was most likely named by Romans from a combination of two words: "praecocia" from Latin, which means "early matured," and "albarquq" from Arabic, which means "short ripening time." It is a temperate fruit that thrives in climates that have distinct seasons. It necessitates a relatively cold winter and reasonably warm spring and early summer temperatures (Ahmadi et al., 2008). The apricot tree is a deciduous tree that needs a cold winter for proper dormancy and flower bud growth (400–600 hours below 7.2 °C). In areas with a subtropical climate, apricot production is not feasible. Apricots, including peaches, plums, cherries, and mangoes, are drupes in which the outer fleshy portion (exocarp and mesocarp) covers a hard stone (endocarp) containing a seed. The color of the fruit varies from orange to

orange red, with some cultivars being cream white to greenish white (Ruiz et al., 2008; Riu-Aumatell *et al*., 2005).

According to Iamanaka *et al*., (2005), apricot samples were not infected by any molds, while black sultanas were contaminated with A. niger, followed by plums, figs, and dates. *A. flavus* was not included in any of the samples, with the exception of white sultanas and Fig.

2-4-4: Walnut(*Juglans regia L*)

One of the finest temperate for nuts is *Juglans regia L*), a member of the Juglandaceae family. It is the world's oldest cultivated fruit, and it grows wild almost everywhere in Turkey (Şen *et al*.,1986; Özkan *et al*.,2005). Oil-producing crop plants are also critical to the agricultural sector's economic development. Protective coatings, dispersants, pharmaceuticals, cosmetics, soaps, and a number of synthetic intermediates as stabilizers in plastic formulations are all made with oilseeds containing rare fatty acids (Hosamani *et al*.,2000; Eganathan *et al*.,2006).

Walnut production is expected to increase as the bearing age of the trees increases, as well as the amount of land planted. 50% of the time. The majority of the output is consumed on-site, with the remainder being sold. The majority of marketed walnuts are eaten whole, with just a small percentage processed (zcan, 2009).

2-5: Detection of OTA(Ochratoxin A)

It has been encouraged to develop methodologies that enable simultaneous extraction of these mycotoxins in various matrices. Analytical methods for extracting and analyzing these two mycotoxins have advanced in recent years (Monaci *et al*., 2004; Tabata *et al*., 2008).

Most validated methods for ochratoxin A extraction were focused on the solubility of these compounds in organic solvents or alkaline solutions [González-Peas *et al*.,2004b], and most of the validated methods were based on the extraction of Ochratoxin A through the solubility of these compounds in organic solvents or alkaline solutions. HPLC with fluorescence detection is the most commonly used technique. To achieve the best detection conditions for each toxin, different excitation and emission fluorescence parameters (OTA 335 and 465 nm; CIT 331 and 500 nm) were used. However, in order to achieve low detection limits and protect the HPLC column, an efficient sample extract clean-up is needed for this analytical process. Solid-phase extraction (SPE) of the sample using an immunoaffinity column is the most common clean-up process. This form of SPE makes the clean-up stage much easier by producing high-purity extracts that can be injected directly into the HPLC column [Entwilse *et al*., 2000; Molinié *et al*.,2005]. and are widely used to determine mycotoxins in a variety of matrices with high analytical performance. However, they have the drawback of requiring unique antibodies for each mycotoxin. There hasn't been an IAC for determining CIT until now. Molinié *et al*.,2005) developed a flexible process for raw cereals and cereal products using partition. It had been announced that new extraction and analysis techniques were being developed. The quechers (Quick, Simple, Cheap, Effective, Rugged, and Safe) Frenichet al.,2011; Fernandes et al.,2013] method was adopted for mycotoxin extraction due to its speed and low cost, and enzyme-linked immunosorbent assays (ELISA) (Klari *et al*.,2009; Bazin *et al*.,2010) are emerging. Their simplification is being furthered by the creation of colored immuno-tests, such as rapid disposable membrane-based assay tests or clean-up tandem immune assay columns. The aim of this study

was to show how extraction pH can trigger OTA content misinterpretation and CIT cross-reactivity on IAC that recognizes OTA. We investigate the explanation for the underestimation of OTA content in wine following a typical anthocyanin removal procedure. For OTA and CIT occurrence in wheat samples, we compare several extraction and clean-up methods (Entwilse *et al*., 2000; Molinié *et al*., 2005).

2-6 Minimizing Ochratoxin A Contamination

Biological options require the use of microorganisms and their enzymes in addition to physical and chemical therapies (Chen *et al*., 2018).

Several microorganisms can inhibit mycotoxin biosynthesis and detoxify mycotoxins once they've been formed, either by binding them to their cellwalls or degrading them into less toxic compounds, or a combination of both (Péteri *et al*., 2007).

Direct application of detoxifying microorganisms to polluted food matrices is the most common solution, but the biological agent must already be recognized as safe for consumption (GRAS) (for example, lacticbacteria and yeasts) (Shetty and Jespersen, 2006).

Microorganisms that can detoxify mycotoxins but not gras are not permitted to be used directly in the food matrix. As a result, looking for enzymes based on their degrading capacities can lead to some interesting application possibilities (Vanhoutte *et al*., 2016).

Some enzymes that are known to be involved in the production of inochratoxin Carboxypeptidases, deoxygenases, lipases, amidases, and proteases are all enzymes that degrade proteins (Vanhoutte *et al*., 2016).

2-7 Actinobacteria strains

Several actinobacteria strains were able to minimize the occurrence of OTA either by preventing its formation, degrading it, or both mechanisms. The methodology employed in this study screening by nine modalities, clustering and correlation analysis) helped easily distinguish the features of each strain and to group them into clusters of comparable abilities. A heatmap representation helped easily select specific strains according to the desired effect. For instance, we could choose strains that strongly inhibit OTA specific production without affecting P. verrucosum growth to preserve the ecological niche, or strains that decreased OTA specific production but had low degradation capacities, if we wish to deepen the study of the inhibition of the mycotoxin biosynthetic pathway. Remarkable observations such as the degradation ability of OTA on the solid medium were very scarce among the strains studied, found for only five strains among the sixty. Interesting correlations such as the increase in OTA specific production when growth was strongly reduced have also been observed. Thus, our collection represents a great start for further studies in order to identify active metabolites and enzymes, and to provide a better understanding of the various interactions that can exist between actinobacteria, *P. verrucosum*, and OTA. Among the three strains studied by HPLC MS/MS for OTA degradation, no common by-products were found (Campos-Avelar *et al*., 2020).

2-8 Pleurotus ostreatus and *Trichoderma harzianum*

Al-mohaidi and saadon., 2020, reported reduction in the level of colonial diameters of Aspergillus niger in which the rate of inhibition reached to 71.79 ± 0.06% at concentration 30% of *P. ostreatus* filtrates and better than the calcium citrate when treating each one separately.

2_9 Prevention and decontamination strategies

To avoid the production of OTA, many crucial points of control in food processing must be considered. For example, cereal drying, processing, and storage, coffee, cocoa, and spice drying, transport, and storage, and grape harvesting and transportation (Bucheli and Taniwaki, 2002; Belli.,2004; Magan and Aldred,2005).

It is not possible to fully eliminate OTA from foods. As a result, the Codex Alimentarius Commission (El Khoury and Atoui, 2010) devised a number of methods to reduce OTA. One of these techniques is used while crops are in the pre-harvest period. It entails maintaining the purity of crops in order to prevent fungi from colonizing them. Mechanical damage and insect attack are, in this sense, the most regulated processes. The removal of polluted crop residues reduces the risk of pollution as well (Magan and Aldred, 2005).

One of the most significant factors in preventing OTA pollution is harvesting goods at their peak maturation level. Furthermore, having an acceptable moisture content in products helps to prevent mechanical damage, and collecting in contamination-free sacks or containers reduces the risk of contamination. Overripe, fermented, damaged, or fallen fruits, for example, must be discarded because they can contain high levels of OTA or harbor OTA-producing fungi that could spread quickly(Bucheli and Taniwaki, 2002) Another appealing technique for preventing OTA development is the use of chemical compounds. Some fungicides are effective against fungal invasion, and Complimentary Contributor Copy Prevention by pre-harvest management practices is the most effective approach for preventing mycotoxins contamination.

However, if contamination occurs or continues after this process, post-harvest procedures must be used to control the risks associated with OTA. Traditional methods for preventing fungal growth in grains include drying them quickly after harvest and keeping them dry. However, (Pardo *et al*.,2005b) indicated that lowering the moisture content of grains to below 0.8 is needed to prevent OTA production by *Aspergillus* section *Nigri species*. After harvest, storage and processing are the main stages where contamination can be avoided or detoxification can be achieved by physical separation or chemical inactivation following particular procedures. When stored grains become colonized by fungal organisms, various techniques such as grain segregation, fumigation, aeration and cooling, product sealing, and atmosphere control become important. These methods are used mostly in tropical and subtropical areas, where storage damage is a significant issue. One of the most important post-harvest phases of food handling is storage. As a result, it must be carried out under strict supervision.

2-10: Environmental factors affecting mycotoxin biosynthesis

2-10-1: Temperature

It's one of the factors that affects fungi's ability to develop and survive in the body, as well as their ability to produce toxins. It also means that it is technically successful in nature, according to chinchin *et al* (2010).

Pull factors are factors that help in the conversion of nutrients, grains, and nuts, as well as the best conditions for grading in different climates (1997, Pitt). *A.flavus* thrives at temperatures ranging from 10 to 45 degrees Celsius and humidity levels of up to 75%. The ideal temperature

and relative humidity for the production of Aflatoxins are 25-30°C and 85 percent relative humidity (FAO, 1998)

When stored in moisture, the fungi grow at 65-70 percent or 85-90 percent during sex. A. flavus can grow with up to 90 percent more moisture, while aflatoxins are 95 percent, 98 percent, and a grade of 25. (Bhatt *et al*., 2010)

2-10-2- PH

The amount of acidity or basicity in a substance or expressed in it is known as the hydrogen function. Hydrogen's negative ion logarithm. One of the factors affecting fungi development is hydrogen ion concentration (Mehra and Jatily 1995). *A.flavus and A.parasiticus* have the ability to expand in a pH range of 1.7 to 9.3. (Gemili). 2014, Ion. As the pH is tailored for the sex, hydrogen is one of the most essential elements in the development of toxins. (International Committee of the Red Cross and Red Crescent Societies, 1996) 3.5 - 8 It ranges from *Aspergillus* 2-14 in terms of aflatoxins production: Conditions of storage

On the one hand, storing commodities in a dry location increases storage capacity while also protecting it from development. Fungi, on the other hand, will develop if the storage process is incorrect or if the humidity level is too high. Factors that promote fungi development, as well as the storage period of factors that must be managed when they are present The use of mycotoxins and the lengthening of storage times make it possible to create favorable conditions for mold growth. The synthesis of at afltoxins (Kaaya *et al*., 2000). Foodstuffs are stored in light or dark environments. If darkness plays a role in the activation of fungi and the development of toxins, storing food in the dark doubles the aflatoxin B1 concentration as compared to light storage (Kuchari and Qattan, 2001).

3. Materials and methods

3-1-1: equipment:

The following equipment which was used in this study listed in table3-1

Table (3-1): equipment used in the laboratory experiments.

No	Equipment	Company	Country
1	Autoclave	Gallen Kaamp	England
2	Benzene burner	Gallen Kaamp	Iraqi
3	Capillary tube	Superestar	India
4	Compound light microscope	Olympus	Japan
5	High Speed Cold centrifuge	Eppendorf	Germany
6	Distiller	Fisons	Japan
7	Electric oven	Memmert	Germany
8	Electrophoresis apparatus	Shandon	England
9	High Speed centrifuge	Hettich	Germany
10	Filter paper	Whatman4	England
11	Gel Electrophoresis	Shndon,scientific	England
12	Hot plate	Gallenkamp	England
13	Incubator	Memmert	Germany
14	Micropipette at different magnifications	Cyan	China
15	PCR thermocycler T100	BioRad	USA
16	Petri dishes	Sailbran	China
17	PH- meter	Philips	Holand
18	Refrigerator	Concord	Lebanon
19	Rotary evaporator	Memmert	Germany
20	Sensitive electronic balance	Gallen Kaamp	England
21	Slides and cover slides	Superestar	India
22	Test tube	Superestar	22
23	TLC	Unisonics	England
24	Ultra violet	Bioneer	Korea
25	Vortex mixer	Talboys	USA
26	Warring blender	National	Japan
27	Water bath	Tafesa	Germany
24	Water distillatory	Gallenkamp	England
25	Camera	Nikon	Japan
26	Cell Disruptor Genie vortex	Scientific Industries	USA
27	Tube Rack Double Panel Microcentrifuge PCR Centrifuge Tube Holder	P-ABC	USA
28	Eppendorf tubes	BioBasic	Canada
29	MiniOpticon Real Time PCR	BioRad	USA
30	PowerPac HC Electrophoresis Power Supply	BioRad	USA
31	HPLC		

3-1-2: Biological and chemicals Materials

The chemicals which were used in this study listed in table (3-2).

Table (3-2):. Biological and Chemicals Materials

No	Chemicals	Manufacture	Origin
1	Absolute ethanol	Fluka	Switzerland
2	Acetic acid	BDH	England
3	Agarose Gel	Biobasic	Canada
4	Chloroform	BDH	England
5	Copper sulphate	Scharlau	Spain
6	Dimethyl silphoxide (DMSO)	BDH	England
7	Ethanol alcohol	BDH	England
8	Ethidium bromide	Biobasic	Canada
9	Ferric chloride	Scharlau	Spain
10	Formalin	BDH	England
11	HCL	BDH	England
12	KCL	Scharlau	Spain
13	Ladder	Bioneer	Korea
14	Mercuric chloride	BDH	England
15	Methanol alcohol	BDH	England
16	Isopropanol	BDH CHEM-LAB	Belgium
17	NaCL	BDH	England
18	NaOH	BDH	England
19	Picric acid	Himedia	India
20	Potassium hydroxide	BDH	England
21	Potassium tartarate	Scharlau	Spain
22	Protinase K enzyme	Bioneer	Korea
23	Stander ochratoxin A	promega	America
24	sulfuric acid	Scharlau	Spain
25	TBE buffer	Biobasic	Canada
26	DNA Marker ladder 1500-100bp	iNtRON	Korea
27	DNA Marker ladder 2000-100bp	Bioneer	Korea
29	Free nuclease water	BioLabs	UK
29	DEPC water	Bioneer	Korea
30	Sodium acetate	CDH	India

3-1-3: Agar Culture Media used in the Study

Table (3-3): Culture media used in the laboratory experiments.

No	Media	Company	Used
1	Brain Heart Infusion Broth (BHI Broth)	Hi Media laboratories Limited	
2	Potatos Dextrose Agar(PDA)	Hi Media laboratories Limited(India)	culture
3	Sabouraud Dextrose Agar Medium(SDA)	Hi Media laboratories Limited	Sub- culture
4	Sabouraud Dextrose Broth	Hi Media laboratories Limited	DNA extraction

3-2: Methods

3-2-2: Agars culture media used in the study: -

3-2-2-1: Potato dextrose agar medium (PDA)

Medium was prepared according to the manufacturer's instructions, by dissolving 39 grams of the medium powder in 1 liter of distilled water, sterilized by autoclave a temperature of 121°c for 20 minutes and then cooled to 45°c , after that added chloramphenicol in concentration of 250 mg /l used the agar for the purpose of isolating.

3-2-2-2: Sabouraud Dextrose Agar medium (SDA)

Medium was prepared according to the manufacturer's instructions, by dissolving 65gm from Sabourauds Dextrose Agar in 1 liter of distilled water, sterilized by autoclave a temperature of 121 °c for 20 minutes and then cooled to 45°c, after that adding chloramphenicol in concentration of 250 mg /l used the agar for the purpose of isolating and diagnosing fungus.

3-2-2-3: Sabouraud Dextrose Broth (Sabouraud Liquid Medium)

Medium was prepared according to the manufacturer's instructions, by dissolving 30gm from Sabourauds Dextrose powder in 1 liter of distilled water , sterilized by autoclave a temperature of 121 °c for 20 minutes and then cooled to 45°C, after that adding chloramphenicol in concentration of 250 mg /l used the agar for the purpose of isolating and diagnosing fungus

3-2-2-4: Brain Heart Infusion Broth (BHI Broth)

Medium was prepared according to the manufacturer's instructions, by Suspend 37 grams of the powder of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution.

3_2_3 Growth and maintenance of fungi

All fungal isolates were re-cultured on PDA, by taking 0.5 cm (diameter) that cut from the age of recent colony of this fungus and placed on the center of Petri plate containing sterilized PDA supplemented with chloramphenicol (0.05 g/l) at pH 5.6 and incubated at 28±1ºC for 7 days and then kept in the refrigerator at 5˚C. Fungi were maintained on PDA slants at 5 °C and subculture were made every 20 days throughout this study.

3-2-3_1: Samples Collection

The Fungi were collected from Hilla City, Babylon Province, Iraq. The fungi associated with dried fruit used in this research were isolated by surface sterilization of the seeds using a solution of sodium hypochlorite at a concentration of 1 % for a minute and then washed with sterile distilled water three times. The seeds were planted in Petri dishes containing PDA medium with 10 seeds in each dish and three Repeats and dishes were placed in the incubator at 25˚temperture for 7 days.

Fungi isolates were purified and diagnosed according to taxonomic and microbiological characteristics depending on the occurrence and frequency were calculated according to the following equations.

3-2-3-2 Dried fruits samples

Between September 2020 and February 2021, five types of dried fruits including raising, Iraqi raisins, fig, apricot, Hazelnut and Common plum were collected from local markets in the city of Hilla, Babylon, Iraq. Samples were stored in plastic bags at −20˚C until the isolation of fungi.

3-2-3-3 Isolation of fungi

Approximately 250 g of each dried fruits (Apricot, Raisins, Iraqi Raisins, Fig and Hazelnut) were selected. The fruits were cut into small segments (3 mm in diameter) with a sterilized blade, surface sterilized in 1% hypochlorite for 2 min, plated on Sabouraud dextrose agar (SDA) aseptically and then incubated at 28°C for 5 days. A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the SDA plates and incubating at 28°C for 5 days.

3-2-3-4 Identification of the fungal genera

Fungal isolates were sub-cultured for purification, and then identified on the basis of their colony morphology and spore characteristics, growth pattern, conidial morphology, and pigmentation (Rajankar *et al*., 2007; Oyeleke and Manga, 2008). The identification of the isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of

lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Onuorah et al.(1988).

3-2-3-5 : Fungi Isolation from Dried fruit

The Fungi associated with dried fruit used in this research were isolated by surface sterilization of the seeds using a solution of sodium hypochlorate at a concentration of 1 % for a 3 minutes and then washed with sterile distilled water three times. The seeds were planted in Petri dishes contained PDA medium with 10 seeds in each dish and in three Repeats and dishes were placed in the incubator at 25˚temperture for a period of 7 days. Fungi isolates were purified and diagnosed according to taxonomic and microbiological characteristics depending on (Moubasher, et al 1993) and (Pitt and Hocking, 2009), the occurrence and frequency were calculated according to the following equations(Al-Ameri *et al.*,2018):

$$\text{Percentage to appearance} = \frac{\text{The number of samples in which the species appeared}}{\text{Total number of this fungal species in all age groups}} \times 100\ (\%)$$

$$\text{Frequencypercentage} = \frac{\text{The number of isolates of one species}}{\text{The total number of isolates of all species}} \times 100\%$$

3-2-4: Diagnosis by Using PCR

3-2-4-1 Primers:

PCR and Real Time PCR primers were designed in this study using NCBI-GenBank database and primer3 plus online. These primers were provided by Macrogen company from Korea as following table (3-4):

3-2-4-2: Molecular study Kits

3-2-4-3: PCR detection Kits

Table (3-4): The PCR detection Kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
1	**G-spin Total DNA extraction kit**	**INtRON**	**Korea**
	CL buffer lysis buffer		
	BL buffer binding buffer		
	Proteinase K		
	WA buffer		
	WB buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
2	**Maxime PCR PreMix kit**	**INtRON**	**Korea**
	Taq DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris-HCl pH 9.0, KCl, & $MgCl_2$		
	Stabilizer and loading dye		

3-2-4-2-4: Methods

3-2-4-2-4-1: Fungal DNA extraction

Fungal genomic DNA was extracted from *A. Ochraceus* isolates by using (**G-Spin DNA extraction kit with modification**) as and done according to company instructions as following steps:

1-Sample Preparation:

a- 1 ml cultured fungal cells were transferred to 1.5 ml microcentrifuge tube then centrifuged at 10000 rpm for 1 minute then the supernatant was discarded.

2-Cell Lysis steps:

a- 180µl GT buffer and 250mg glass bead were added to the tube and the cell pellet suspended by vortex, then 20 µl of Proteinase K was added and the mixtures were incubated at 60ºC for 30 minutes. During incubation periods the mixtures tubes were inverted every 3 minutes.

b- 200µl GB buffer were added to each tube and mixed by vortex for 10 seconds. Then the tubes incubated at 60ºC for 10 minutes with inverted the tubes every 3 minutes through incubation periods.

3-DNA binding steps:

a- 200µl absolute ethanol were added and immediately mixed by vortex, then precipitates if happen was broken by pipetting.

b- A GD column was placed in a 2 ml collection tube and all mixtures were transferred (including any precipitate) to the GD column. Then centrifuged at 10000 rpm for 1 minute. The 2 ml collection tubes that contains the flow-through were discarded and placed the GD column in a new 2 ml collection tube.

4-Washing steps:

a- 400µl W1 buffer were added to the GD column, then centrifuged at 10000rpm for 1 minute. The flow-through was discarded and placed the GD column back in the 2 ml collection tube.

b-600µl Wash Buffer were added to the GD column. Then centrifuged at 10000 rpm for 1 minute. The flow-through was discarded and placed the GD column back in the 2 ml collection tube. and the tubes were centrifuged again for 2 minutes at 12000 rpm to dry the column matrix.

5-Elution steps:

a- The dried GD column was transferred to a clean 1.5ml microcentrifuge tube and 100µl of pre-heated elution buffer were added to the center of the column matrix.

b- The tubes were let stand for at least 3 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 1 minutes to eluted the purified DNA.

3-2-4-2-4-2: Estimation of extracted total DNA:

The extracted total DNA was checked by using Nanodrop (Thermo Scientific NanoDrop Lite UV Visible Spectrophotometer. USA) that measured DNA concentration (ng/µL) and checked the RNA purity at absorbance (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).

2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipetted 2µl of free nuclease water and placed onto the surface of the lower measurement pedestals for blank the system.

3. The Nanodrop sampling arm was lowered and 1µl DNA sample measured.

3-2-4-2-4-3: PCR master mix preparation

PCR master mix reactions for all genes was prepared by using (Maxime PCR PreMix kit) and this master mix done according to company instructions as following table (3-5):

Table (3-5): Standard PCR master mix protocol:

PCR Master mix	Volume
DNA template 5-50ng	5µL
Forward primer (10pmol)	2µL
Reveres primer (10pmol)	2µL
PCR water	13µL
Total volume	20 µL

After that, these PCR master mix components that mentioned in table above placed in standard Maxime PCR PreMix kit tubes that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$, and loading dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, and then placed in T100 PCR Thermocycler (BioRad-USA).

3-2-4-2-4-4 : PCR Thermocycler Conditions

PCR thermocycler conditions protocol for each gene was calculated by using **Optimase ProtocolWriter™** online application and done by using convention PCR thermocycler following table (3-8):

Table (3-6): PCR thermocycler conditions protocol:

No.	Gene name	Initial denaturation Temp./time	Denaturation Temp./time	Annealing Temp./time	Extension Temp./time	Cycle	Final extension Temp./time	Hold Temp./time
3	18SrRNA gene ITS2	95°C/ 4 min	95°C/ 30 sec	59°C/ 30 sec.	72°C/ 60sec.	32	72°C/ 5min	4°C/forever

3-2-4-2-4-5: PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis method as following steps:

1- 1.5% Agarose gel was prepared in using 0.5X TBE and dissolving in microwave for 5 minutes, and left to cool for 50°C.

2- Then 3µl ethidium bromide stain was added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position and left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.

4- The gel tray was fixed in electrophoresis chamber and filled by 0.5X TBE buffer.

5- 10µl PCR product were loaded in to each well with added 5µl (DNA marker Ladder) in first well. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- PCR products were visualized by using UV Transilluminator.

3-2-5: Test The Ability of *A.niger* and *A. ochraceus* Strains to Produce Ochratoxin A

3-2-5-1: Development of *A.niger* and *A. ochraceus* Strains

The Fungus Strains were purified on a sterile PDA media, the discs from the strains were planted with a diameter of 5mm and with 3 replicates per strain, and these discs were placed in the center of the dishes, after which they were incubated at 30˚c for 10 days.

3-2-5-2: Ochratoxin A Extraction

The Ochratoxin A was extracted from *A.nige*r and *A. ochraceus* according to Macdonald et al.,(1999), where three dishes were taken from the growth Fungi for a week at a temperature of 30˚C and cut the grown medium of Fungi in each plate and put these pieces in an electric blender and add 100 ml of a mixture consisting of water and chloroform in a ratio of 1:1, after which the mixture was filtered by a 4 layers muslin cloth and then transfer the filtrate to a separating funnel and shake well, then take a chloroform layer and concentrate it by placing it in the electric oven at 45˚C.

3-2-5-3: Determination of Ochratoxin A in *A. niger* and *A. ochraceus*

3-2-5-3-1: Thin Layer Chromatography

The TLC technique was used to detect the ability of *A.niger* and *A. ochraceus* strains. A 9:1 methanol: distilled water separation system used. A light straight line was made on the TLC plate 1.5 cm away from the base of the lower plate and then 1.5 µl of the standard *ochratoxin A* was applied by a capillary tube on the line. On the left side, the same amount of each sample was placed to the right of the standard *Ochratoxin* A After that, the spots were left to dry, then the TLC plates were placed in separation basin containing a mixture of methanol and distilled water 9:1 respectively and monitored until the mobile phase reached a distance of 1.5 cm from the upper end of the plate. The plate was left to dry and then

examined under ultraviolet ray with a wavelength of 365nm. The revealed the presenced of the toxin by matching the location and color of the fluorescent of the extract with the location and color of *Ochratoxin A and Rf ratio* calculated by (Sobolev and Dorner, 2002).

3-2-5-3-2: High Performance Liquid Chromatography

Ocratoxine was extracted from samples (5ml or 5gm) by homogenization with 20 mL acetonitrile: H2O (6:4, v=v) for 2 minutes. The extract was filtered and 4 mL of filtrate was diluted with 25mL phosphate buffer saline pH 7.4 (PBS). The samples were degassed in a sonic bath for 30 minutes, then the pH was adjusted to 7.2 using 2 M sodium hydroxide5 mL of acetonitrile is added to the sample and then stored until the analysis is performed.

3-2-6: Evaluation of VC-Mediated Inhibition Between *P. ostreatus* or *T. harzianum* against *A. niger* and ***A. ochraceus* species.**

1- Sandwiched Petri plates trechnique (volatile Interaction Assays)

Sandwiched Petri plates, a setup described in Dennis and Webster (1971) with modifications, was employed to determine if and how *T. harzianum* and or *P. ostreatus* VCs affect the growth of A. niger and A. ochraceus. Both *A. niger* and *A. ochraceus* was individually placed on top of *T. harzianum* and *P. ostreatus* Petri plates (9 cm diameter). Plates were sealed with three layers of Parafilm, and incubated at at 26 ± 2 °C. Each plate of pathogenic fungi also was sandwiched with an un-inoculated PDA plate (control treatment). Colony diameter of pathogenic fungi was measured 5 days later. The inhibitory effect of antagonistic fungi VCs on Trichoderma in the same way except that 5-day-old (after the inoculation of culture plug). Culture of pathogenic fungi was used to ensure enough

biomass. Colony diameter of *T. harzianum* and *P. ostreatus* was measured 36 h later.

3-3:In Vitro Antagonist Assay

To determine the antagonistic potential of bioagent fungi against fungal pathogens isolates (*Aspergillus niger* and *A. ochraceus*), dual culture method (Kuzmanovska *et al*., 2018) was used in this study. Mycelial disc (5 mm diameter) taken from the margin of the 5-d old culture of each pathogen isolate was placed 1.5 cm away from the periphery of the Petri plate (90 mm) and a disc with the same size of each tested bioagent fungi was placed in the same manner but on the opposite end of pathogen sample. Control plates were maintained for each pathogen. Three replicates of the experiment were carried out. These plates were incubated at 26 ± 2 °C, after 7 d of incubation,

The per cent growth inhibition (PGI) was calculated at the seventh day using the following formula: where PGI = Growth inhibition of pathogen (%); C = Radial growth of the pathogen (control); T = Radial growth of the pathogen (treated).

$$PGI = [(C - T)/C \times 100)]$$

3_4 Effect of Non-Volatile Metabolites (NVM) filtered from bioagent fungal cultures.

Preparation of *T. harzianum* and *P. ostreatus* filtrate

The methodology described by Dennis and Webster1971; Abd El-Hai and and Ali, 2019 was used to evaluate the potential of NVM (filtered from cultures) produced by *T. harzianum* and *P. ostreatus* isolates against plant pathogenic fungi (*Aspergillus niger* and *A. ochraceus*). Both bioagents and pathogenic fungi were grown on plastic Petri dishes (9 cm diameter containing Potato-Dextrose-Agar (PDA)

medium. Incubation of the cultures occurred at 26±2 ºC for seven days. To obtain the liquid phase with the non-volatile metabolites, the *T. harzianum* and *P. ostreatus* isolates were cultured in bottles 50 ml size containing 25 ml sterile PDB by cutting 0.5 mm culture Dicks 5 days' old followed by incubation at 26 ± 2°C on the shaker at 140 rpm in the dark for 10 days. The fungal culture was filtrated twice through filter paper No.1, then centrifuged and sterilized using membrane filter of pore size (0.22 μm). The resulted filtrate was kept under cooling in the refrigerator in a dark bottle till use.

3-5:Effect of Non-Volatile Metabolites (NVMs) on growth of pathogenic fungi

Filtrates of both fungi were used as antifungal agents in this study. Three replicates were prepared with agar discs (5 mm diameter) taken from pathogen cultures. The mycelial agar disks were deposited in the center of each Petri dish containing PDA medium, supplemented with the respective antagonist culture filtrates (25%). Control plates consisted of mycelial agar disks of each pathogen deposited in PDA medium, with sterile distilled water added. Radial mycelial growth of the pathogen was carried out by taking the measurements of the diameter of the colonies, in centimeter. These measurements were used to calculate the inhibition Index of Mycelial Growth (Menten et al. 1976), using the equation: IMG (%) = $[(D_{ctreat} - D_{treat}) / D_{ctreat}]$ x100, where D_{ctreat} = diameter of the radial mycelial growth of the pathogen in the control treatment without filtrates; D_{treat} = diameter of the radial mycelial growth of the pathogen in the treatment with the filtrates. These evaluations were performed when the entire surface of the medium, in the control treatment, was colonized by the pathogen.

3-6:Statistical analysis

The data were expressed as mean ± standard error (SE). Statistical analysis was carried out using factorial experiments and using least significant difference (LSD) to compare between two means at significant level $p \leq 0.05$.

4-1: Isolation and Morphological Identification of fungi

Colony the color, periphery of the fungal colony, texture, and colony reverse colors of several fungal species isolated from dried fruit samples exhibited substantial heterogeneity in morphological and cultural traits. As shown in Figure (4-1), ten species were identified based on morphological characteristics, four of which are *Aspergillus* species: *A. niger, A. terrus, A. flavus, and A. ochraceus. 380* fungal isolates were counted on PDA in raisins, raisins Iraq, figs, apricots, Hazelnut and Common plum samples distribution of fungal contaminated dried fruits, according to the fungal analysis of the dried fruits samples. *Aspergillus sp. ,Fusarium* sp. , *Nigrospora spp*. and *Penicillium spp*. were among the isolated species.

Aspergillus spp. was the most prevalent genus, as indicated in Figure (4-1). The results of this experiment (Table4-2) showed that the total number of isolates was 380 isolates. The highest number of isolates was obtained by *A. niger* 80 (21%) followed by *Fusarium sp*. 53 (13.9%) then, *A.flavus* 55 (14.47%), *Penicillium spp.* 34(8.9%), *A. terrus* 50 (13.1%), *A. ochraceus* 40 (10.5%) and *Nigrospora sp.*25 (6.5%) respectively.

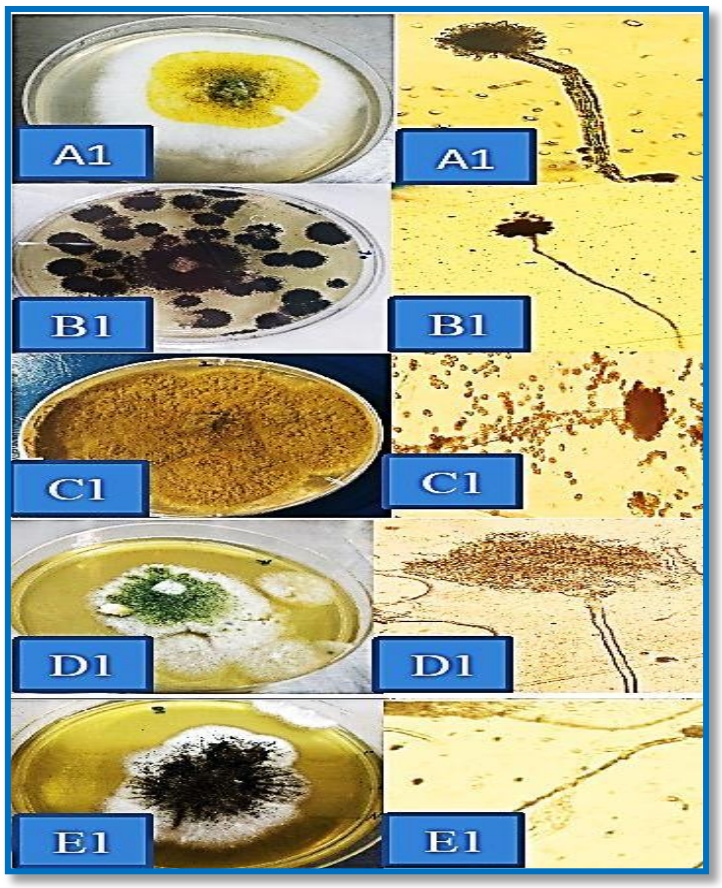


Figure 4-1. Photographs of the colonies of *A. ochraceous(*A1), *Aspergillus niger* (B1), *A. flavus* (c1),*A. terreus* (D1) and *Nigrospora* (E1),in PDA at 25 ºC after 7 days

Table4-1. Number of fungi isolates from dried fruits

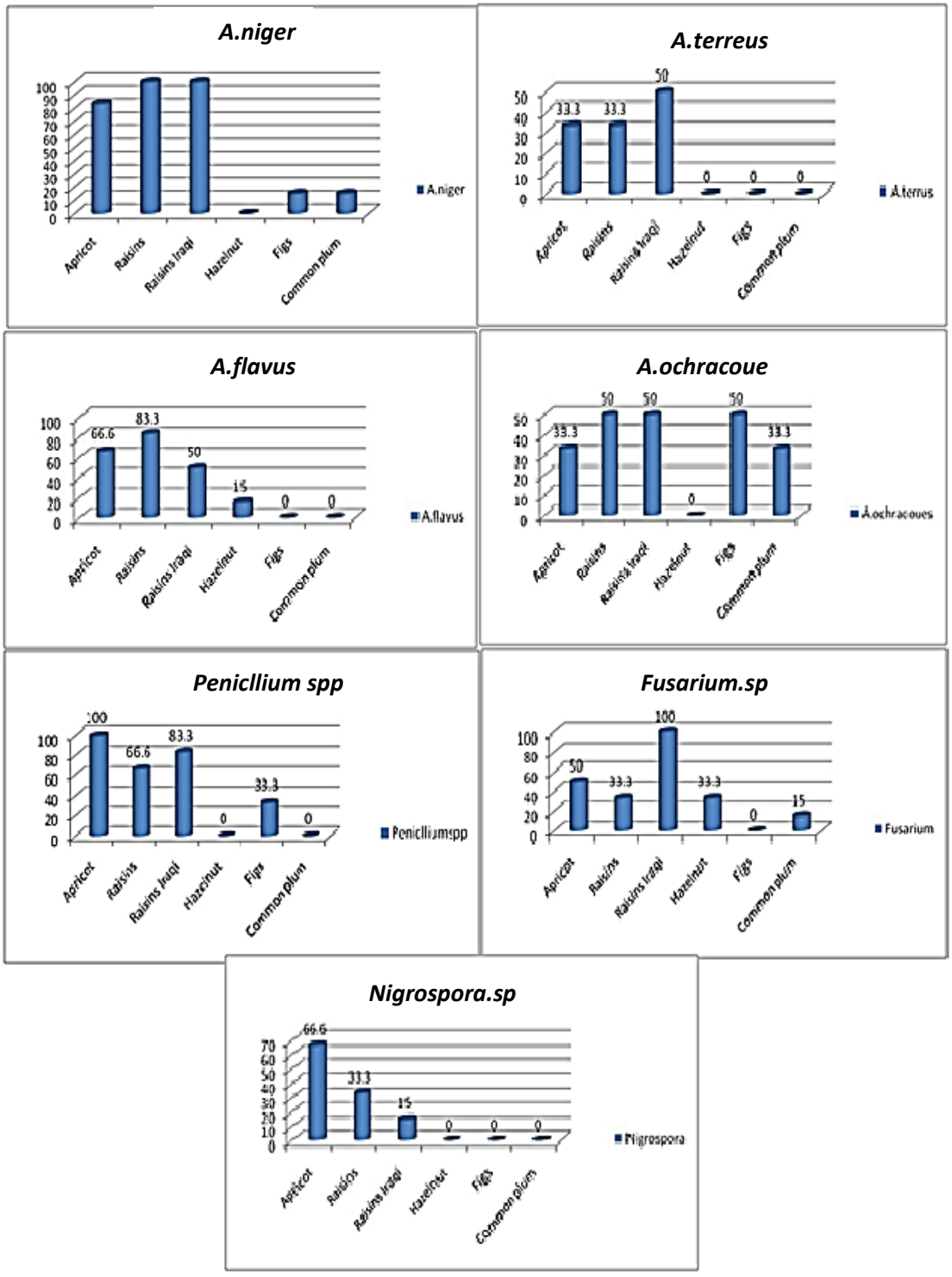
Fungal species	Number of isolates	Proportion of each isolate %
A Aspergillus. Terreus	50	13.16
A. flavus	55	14.47
A . niger	80	21.05
A. ochraceus	40	10.53
A.Parasiticus	20	5.26
Fusarium sp.	53	13.95
Mucur	10	2.63
Nigrospora sp.	25	6.59
Penicillium spp.	34	8.95
Rhizophs	13	3.42
Total	380	%100

4-2: The Percentage of the Appearance

The percentage of contaminating fungi that appeared on dried fruits varied depending on the type of dried fruits and the contaminant fungus. Apricot, Raisins, and Iraqi Raisins all had rather uniform infection to the dried fruits. While infection in other dried fruits (Hazelnut, Figs, and Common plum) was variable (Table 4-3). As described below:

The proportion (%) of *A. niger*, *A. terreus, A. flavus*, *A. ochraceus*, *Fusarium spp., Nigrospora isolated* from dried fruits (Vitis vinifera L. ,ficus Carica L. , prunus armeniaca L. , *Juglans regia L* and Common plum) grown on PDA for seven days at 25±1 ^{o}C are as the following: Abricot: *A. niger* (83.3%), *A. terreus* (33.3), *A. flavus*, (66.6), *Fusarium* spp. (50), *Nigrospora* (66.6), *Penicillium spp*. (100) and *A. ocraceus* (33.3). *Raisins:A. niger* (100%), *A. terreus* (33.3), A. *flavus,* (83.3), *Fusarium spp.* (33.3), *Nigrospora* 33.3), *Penicillium* spp. (66.6) and *A. ocraceus* (50).

Iraqi raisins: A. niger (100%), *A. terreus* (50), *A. flavus*, (50), *Fusarium spp*. (100), *Nigrospora* (15), *Penicillium* spp. (83.3) and *A. ocraceus* (50). Hazelnut: *A. niger* (0), *A. terreus* (0), A. *flavus,* (15), *Fusarium spp*. (33.3), *Nigrospora* (0), *Penicillium spp*. (0) and *A. ocraceus* (0).*Figs: A. niger* (15), *A. terreus* (0), *A. flavus*, (0), *Fusarium spp.* (0), *Nigrospora (0), Penicillium spp.* (33.3) *and A. ocraceus (*50). *Common plum: A. niger (*15*), A. terreus* (0), *A. flavus,* (0), *Fusarium spp*. (15*), Nigrospora* (0), *Penicillium spp.* (0) *and A. ocraceus* (33.3).


A.niger
A.niger
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
A.terreus
A.terrus
33.3
33.3
50
0
0
0
A.flavus
A.flavus
66.6
83.3
50
15
0
0
A.ochracoue
A.ochracoues
33.3
50
50
0
50
33.3
Penicllium spp
Penicllumspp
100
66.6
83.3
0
33.3
0
Fusarium.sp
Fusarium
50
33.3
100
33.3
0
15
Nigrospora.sp
Nigrospora
66.6
33.3
15
0
0
0

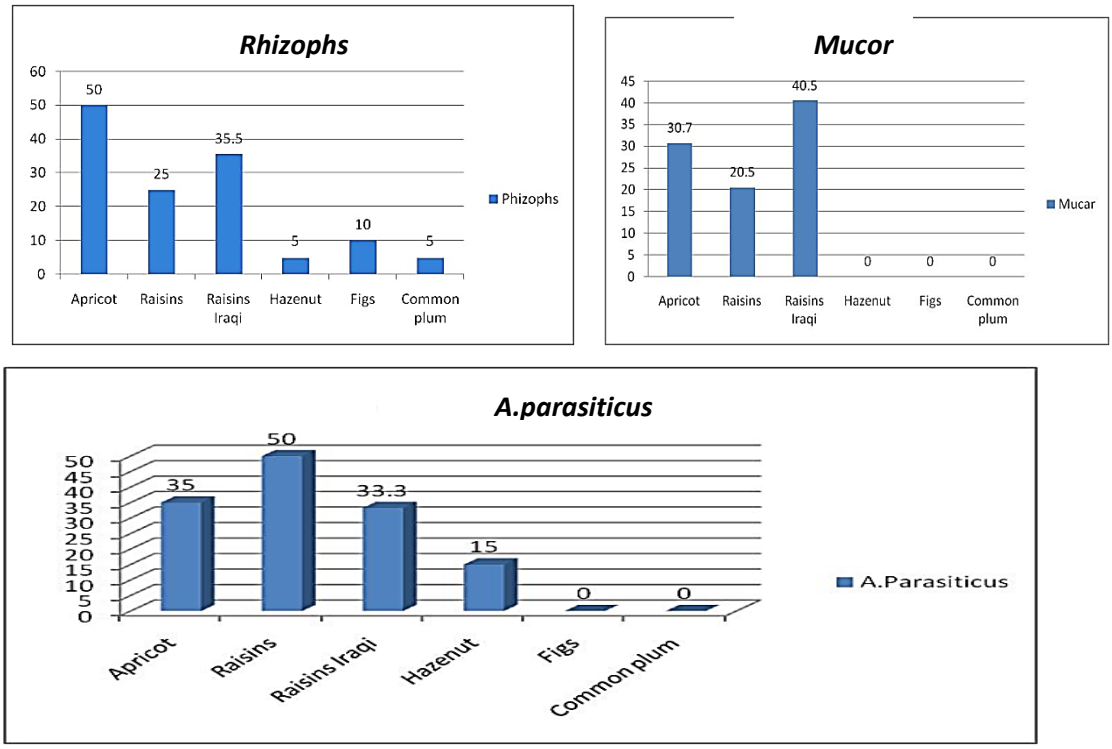
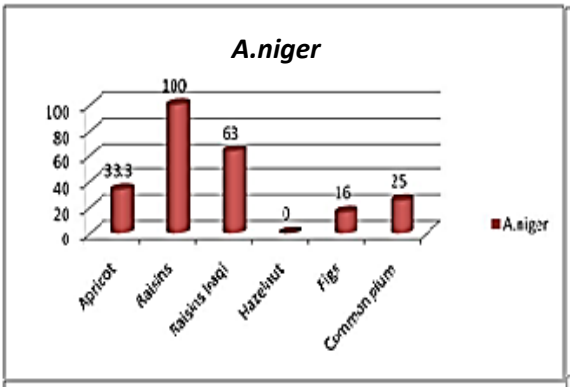


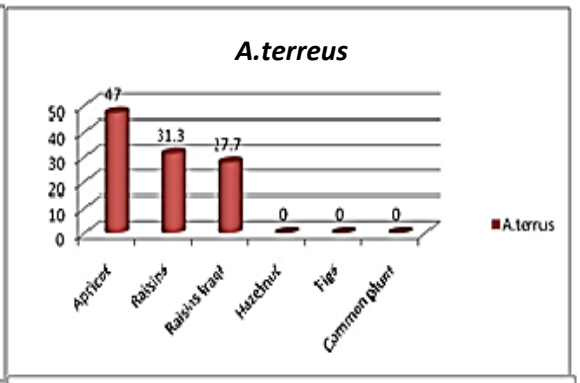
Figure (4-2): The percentage of the appearance for *A. niger*, *A. terreus*, *A. flavus*, *A. ochraceus Penicillium spp*., *Fusarium* sp. , *Nigrospora sp.* , *Rhizophs*, *mucor* and *Paraciticus* isolated from dried fruits (raisins, Iraq raisins , figs, apricots, Hazelnut and Common plum), grown on PDA for seven days at 25±1 ^{o}C.

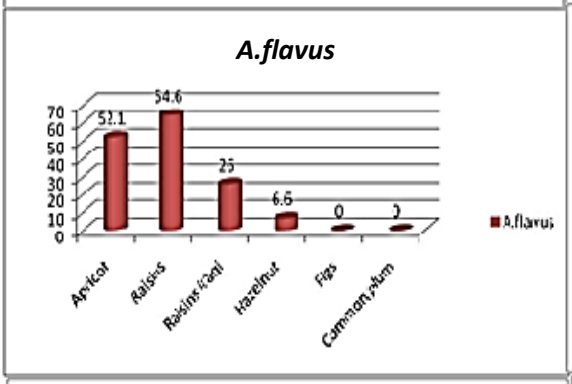
4-3:The percentage of frequency

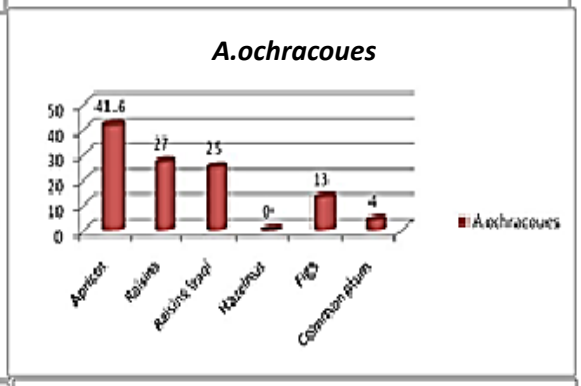
The frequency of *A. nige*r in apricot (33.3%), raisins (100%), Iraqi raisins (63%), Hazelnut (0%), fig (16%) and Common plum (25%). While the Frequency percentage of A. *terreus* to the dried fruits as the following: apricots (47%), raisins (31.3, and Iraqi raisins (27.7%) but no infection was reported in the other dried fruits (Hazelnut, Figs and Common plum). But the frequency occurred by *A. flavus* was in apricot (52.1%), raisins (64.6%), Iraqi raisins (26%), Hazelnut (6.6%), fig (0%) and Common plum (0%). In contrast, the the percentage of frequency in dried fruit happen by Fusarium was in apricot (86.6%), raisins (40%), Iraqi raisins (100%), Hazelnut (13.3%), fig (0%) and Common plum (6.6%). Also, the frequency tookplace by *Nigrospora sp*. was in apricot

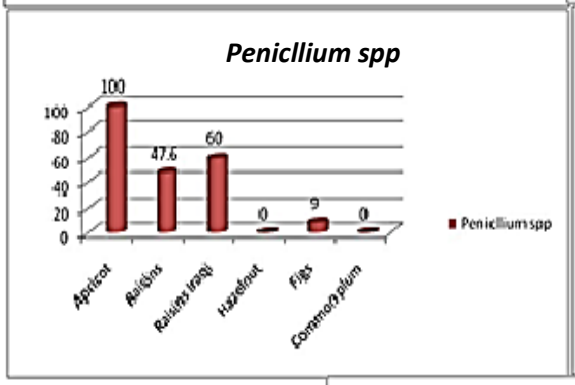
(60%), raisins (33.3%), Iraqi raisins (13.3%), Hazelnut (0%), fig (0%) and Common plum (0%). In dried dried fruits infected by *Penicillium spp*. was as the following: apricot (100%), raisins (47.6%), Iraqi raisins (60%), Hazelnut (0%), fig (9%) and Common plum (0%). whereas in *A. ochraceus*: apricot (41.6%), raisins (27%), Iraqi raisins (25%), Hazelnut (0%), fig (13%) and Common plum (4%).(Saadullah and Abdullah,2014) and that the reason for the emergence of *Aspergillu*s in foodstuffs is the possession of this type of dried fruit with the ability to produce a large number of enzymes that degrade foodstuffs, which are used for nutrition and growth. Low humidity plus the relative density of your sinks, Eaton & Groopman

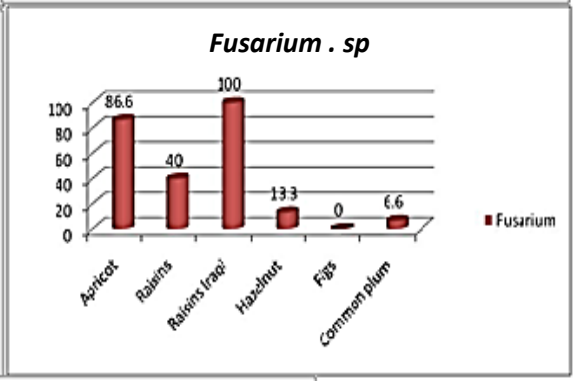

A.niger
100
63
33.3
16
25
0
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
A.niger

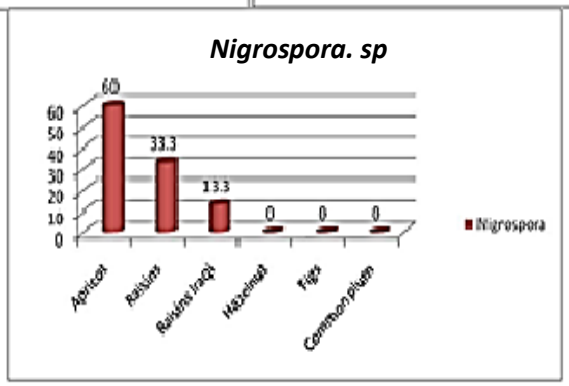

A.terreus
47
31.3
17.7
0
0
0
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
A.terrus

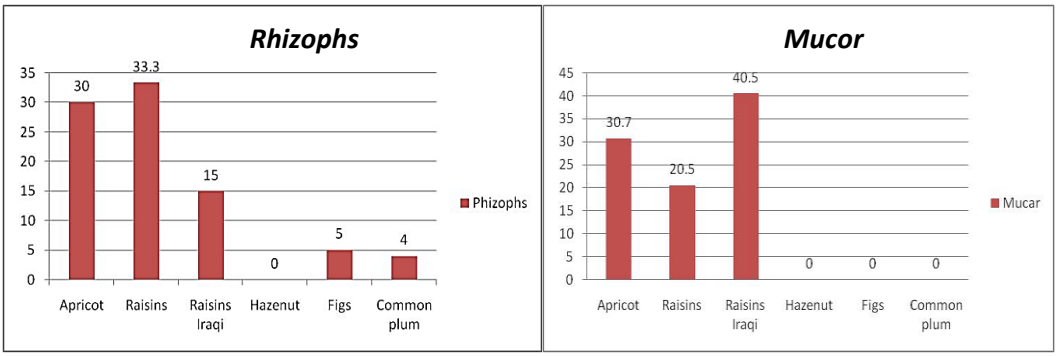

A.flavus
54.6
52.1
25
6.6
0
0
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
A.flavus


A.ochracoues
41.6
27
25
0
13
4
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
A.ochracoues


Penicllium spp
100
47.6
60
0
9
0
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
Penicllium spp


Fusarium . sp
86.6
40
100
13.3
0
6.6
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
Fusarium


Nigrospora. sp
60
33.3
13.3
0
0
0
Apricot
Raisins
Raisins Iraqi
Hazelnut
Figs
Common plum
Nigrospora



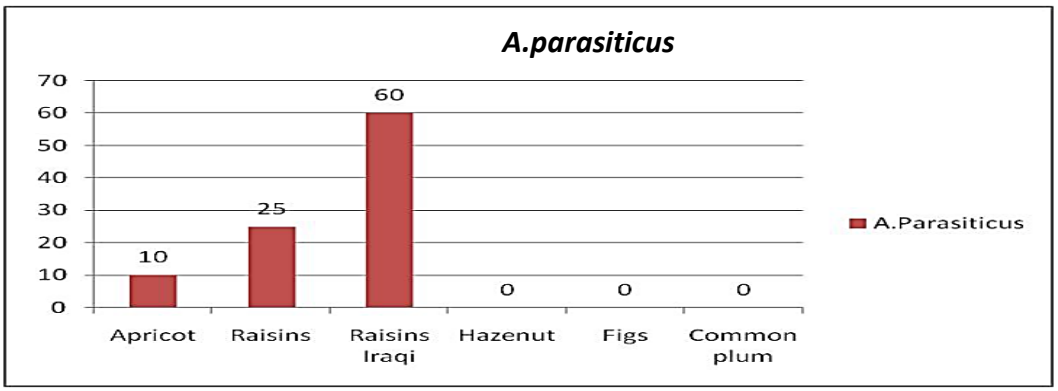


Figure (4-3): The frequency of *A. niger*, *A. terreus*, *A. flavus*, *A. ochraceus Penicillium spp.*, *Fusarium sp.* ,*Nigrospora sp* , *Rhizophs*,*mucar* and *Paraciticus* isolated from dried fruits (raisins,Iraq raisins , figs, apricots, Hazelnut and Common plum), grown on PDA for seven days at 25±1 ^{o}C.

Table(4-2): Frequency and Appearance Ratio of Dried Apricot Fruits

Fungus	Al Hashimih	Al Hilla center	Al Hamzah
	Mean±S.E		
Aspergillus.flavus	28.27±1.81	26.15±4.50	24.70±2.81
A.niger	13.39±4.40	9.30±2.45	9.08±2.69
A.ochracoues	0.00±0.00	1.39±1.38	4.32±2.26
A.terrus	15.47±2.97	23.14±2.31	22.64±0.82
Fusurium Sp.	4.17±2.08	4.42±2.62	1.59±1.58
Mucor	4.17±2.08	1.85±1.85	2.87±1.45
Nigrospora Sp.	3.21±2.09	10.56±2.52	3.03±1.57
Parasiticus	5.52±1.19	6.01±1.22	12.27±1.84
Penicilium Spp.	11.01±3.65	20.16±2.49	18.02±1.11
Rhizophs	6.25±1.60	2.78±2.77	3.34±1.67

LSD(0.05) for fungus=3.576, LSD(0.05) for S=1.959, LSD(0.05) for fungus*S =6.194, S=least significant differences(LSD)

Table (4-3). Frequency and appearance ratio of dried figs

Fungus	Al Hashimih	Al Hilla Center	Al Hamzah
	Mean±S.E		
Aspergillus.flavus	18.18±4.00	26.62±1.95	23.16±3.53
A.niger	14.14±2.08	12.55±1.73	12.18±3.01
A.ochracoues	4.32±1.22	1.51±1.51	4.03±0.41
A.terrus	19.30±1.79	19.21±1.10	14.57±3.74
Fusurium sp.	2.75±1.39	6.35±0.79	5.05±1.60
Mucor	7.63±2.04	4.63±1.31	5.18±1.69
Nigrospora sp.	12.85±3.77	3.97±1.09	9.39±2.95
Parasiticus	3.08±1.63	7.25±1.56	4.10±0.34
Penicilium spp.	9.48±2.98	10.09±1.89	12.11±1.23
Rhizophs	8.25±2.18	4.69±1.74	10.23±1.34

LSD(0.05) for fungus=3.945, LSD(0.05) for S=2.160, LSD(0.05) for fungus*S =6.833, S=least significant differences (LSD)

Table (4-4): The percentage of appearance of the dried fruits of Iraqi raisins

Fungus	Al Hashimih	Al Hilla Center	Al Hamzah
	Mean±S.E		
Aspergillus.flavus	22.44±2.45	20.99±5.75	18.69±0.40
A.niger	12.09±3.72	8.85±1.90	8.72±1.24
A.ochracoues	1.28±1.28	3.34±0.67	3.16±0.80
A.terreus	22.08±2.02	14.15±2.05	16.48±0.62
Fusurium sp.	5.69±1.69	1.59±0.50	5.53±1.36
Mucor	5.46±1.11	3.92±0.92	7.87±0.99
Nigrospora sp.	8.37±0.40	7.09±1.60	7.24±1.07
Parasiticus	5.46±1.11	12.87±3.56	15.07±2.41
Penicilium spp	15.06±0.74	11.98±3.08	16.66±1.08
Rhizophs	4.18±0.20	10.06±2.49	9.55±1.84

LSD(0.05) for fungus=3.575, LSD(0.05) for S=1.958, LSD(0.05) for fungus*S =6.193, S=least significant differences(LSD)

Table (4-5): Frequency and appearance ratio of dried fruits for raisins

Fungus	Al Hashimih	Al Hilla Center	Al Hamzah
	Mean±S.E		
Aspergillus.flavus	34.87±7.43	18.12±2.29	10.14±3.00
A.niger	15.49±3.37	10.14±2.24	12.96±3.76
A.ochracoues	5.28±0.79	4.01±0.37	0.00±0.00
A.terrus	19.36±2.78	18.82±2.64	27.00±4.24
Fusurium sp.	6.42±1.65	5.64±1.88	3.67±1.88
Mucor	4.10±0.76	2.42±0.21	5.63±0.44
Nigrospora sp.	12.79±1.71	5.20±1.01	8.50±2.59
Parasiticus	3.27±0.22	7.23±2.12	4.59±0.80
Penicilium spp	15.04±0.29	13.82±1.82	16.69±2.03
Rhizophs	7.49±0.74	14.35±4.04	12.32±1.76

, LSD(0.05) for fungus=6.844, LSD(0.05) for S=3.748, LSD(0.05) for fungus*S =11.854, S=least significant differences(LSD)

Table(4-6): Frequency and appearance ratio of dried fruit of walnut

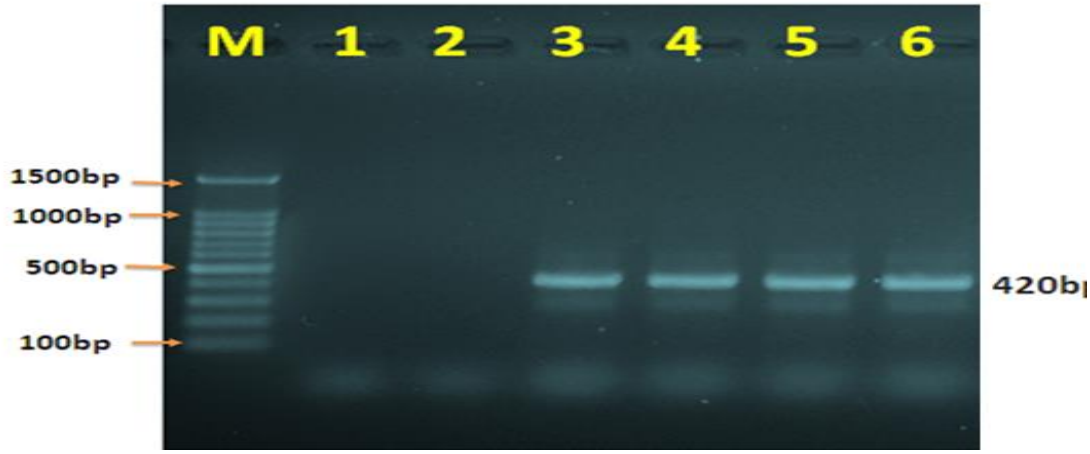
Fungus	Al Hashimih	Al Hilla Center	Al Hamzah
	Mean±S.E		
Aspergillus A.flavus	17.13±2.81	9.70±2.25	15.58±4.32
A.niger	9.66±2.05	14.83±2.43	7.14±1.12
A.ochracoues	0.00±0.00	0.00±0.00	0.00±0.00
A.terrus	13.42±1.66	13.67±2.07	17.96±2.06
Fusurium sp.	4.95±1.71	16.33±3.51	2.38±0.31
Mucor	5.40±1.47	10.03±1.01	7.77±1.16
Nigrospora sp.	13.42±1.66	9.97±1.51	10.17±2.20
Parasiticus	5.24±1.08	9.42±2.42	7.79±0.65
Penicilium spp.	8.02±0.31	7.41±1.40	7.79±0.65
Rhizophs	11.72±3.39	15.97±2.99	18.61±4.33

LSD(0.05) for fungus=6.088, LSD(0.05) for S=3.334, LSD(0.05) for fungus*S =10.545, S=leat significant differences(LSD)

4-4: Polymerase Chain Reaction (PCR)

Aspergillus species have been found to produce OTA, a mycotoxin with nephrotoxic,immunosuppressive, teratogenic,and carcinogenic properties. These data are similar to those Sambrook, and Russell, (2001). After phenotypic and microscopic diagnosis, molecular diagnosis is made using pcr technique As it is a modern and developed technology and one of the most sensitive and accurate techniques that depends on DNA as amplification by means of it facilitates the identification of the diameter (Edel,V. 2000 ;Moslem,et al., 2010)

These results are similar to what they reached (*Patiño et al.,2005) he initiator pcr corelated appropriately with the target genotype and gave positive result Pe-1, Pe-2, Pe-3,Pe-4 ,Pe-5, Pe-6,Pe-7, Pe-8. In 420pb.*



Figure(4-4). Agarose gel electrophoresis image that showed the PCR product analysis of 18S rRNA gene ITS2 gene from extracted DNA of *Aspergillus ochraceus* isolates. Where M: marker (1500-100bp) and the positive *Aspergillus ochraceus* isolates were showed at (420bp) PCR product

4-5: Thin layer chromatographic (TLC)

The results of detection using TLC showed that 6 isolates for each of the *A.Ochraceus* and A.niger were mostly produced ochratoxin in different types of dried fruits of figs, raisins, apricots, Iraqi raisins and dried apricots, and 90% produced the toxin by comparing the color of shine with the extract of each isolate. First sample there is no quantity for Ochratoxin, sample carry over factor is 32.35 in sample 3, 4 there is another toxin in sample 5 and carry over factor is 31.76. In Sample No. 6, the carry-over factor was 32.94.

Table(4-7): RF value of (OTA) detected by TLC for *A.ochracoues* and *A.niger*

Fungus	Isolated No	RF %
A.ochracoues	1	-
	2	32.35
	3	-
	4	-
	5	31.76
	6	32.94
A.niger	1	-
	2	30
	3	30
	4	-
	5	37.4
	6	35.9

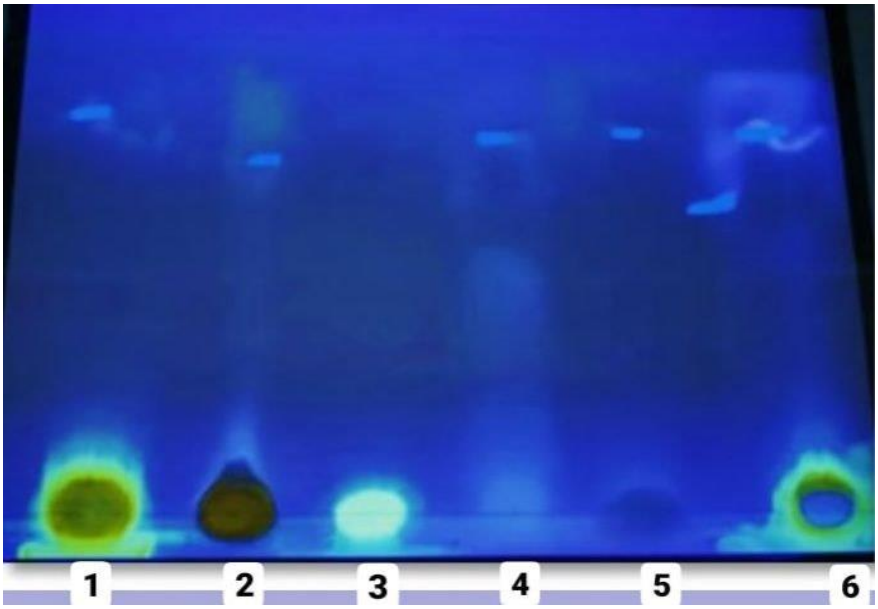


Figure4-5. Thin layer chromatographic (TLC) of Ochratoxin in *A. ochraceus* and *A.niger*

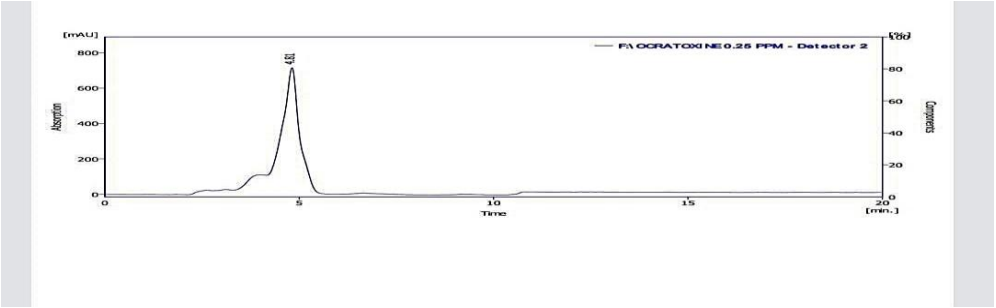
4-6:HPLC Analysis

Under chromatographic settings, appropriate OTA (Figure 3) in *A. ochraceus* extracted from raisin sample was detected using HPLC in a stationary phase and a step gradient polarity system of mobile phase.

It has appeared that the retention time min was (4.81). While the OTA in the fig was (4.86). Similar results were obtained with other studies Al-Hazmi, (2010). The *Ochratoxin* A HPLC chromatogram of the standard appearance of retention time min (0.236) Other studies confirm the presence *Ochratoxin* A in raisin. *Ochratoxin* A in *A.niger* compound in the apricot. a raisin in Iraq, nut a dry fruit sample was identified under the chromatographic conditions Figure (4-4), appearance the maximum of retention time min (4.86) Ochratoxin A in Ochracuse compound in the fig dry fruit sample was identified under the chromatographic conditions f. plants like the result (Chebil,2020). The Ochratoxin A HPLC chromatogram of the standard (Fig4) and the positive sample of the maximum concentration (2.887)

Table (4_8):Standard Ochratoxin A (0.25ppb)

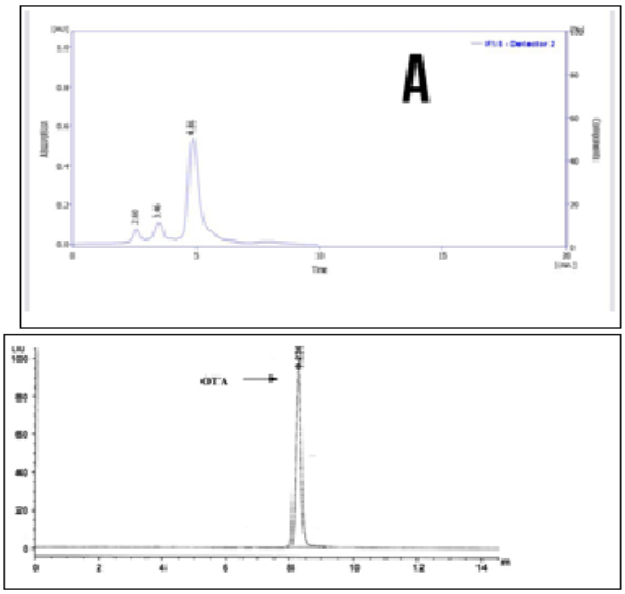
	Eeten.Time (min)	**Area (Mau.s)**	**Height (mAU)**	**Areea (%)**	**Height (%)**	**W05 (min)**	
1	4.813	327.901	64.091	100.0	100.0	0.09	Compound Name
	Total	327.901	64.091	100.0	100.0		



Figure(5_6): HPLC chromatogram standard Ochratoxin A (0.25 ppb)

Table (4-9):HPLC Ochratoxin A concentration in A.niger and A.Ochraceus

No.	Con (ppb)	No.	Con (ppb)
1. A.niger	**2.49**	***4. A.niger***	**2.10**
2. A.niger	**3.14**	***5. A.Ochraceus***	**88.47**
3. A.niger	**2.16**	***6. A.Ochraceus***	**96.79**



Figure(4-7): HPLC chromatogram of Ochratoxin A in raisin contaminated with *A. ochraceus*

Table(4-10): HPLC chromatogram of Ochratoxin A in raisin contaminated with *A. ochraceus*

Reten. Time	Area	Height	Area	Height	W05
[min]	[mAU.s]	[mAU]	[%]	[%]	[min]
2.597	305.873	32.834	5.7	9.3	0.16
3.460	395.666	32.456	7.3	9.2	0.22
4.863	4691.075	285.938	87.0	81.4	0.29
Total	5392.614	351.229	100.0	100.0	

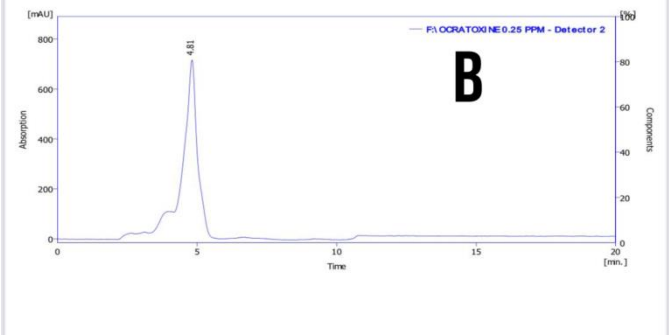
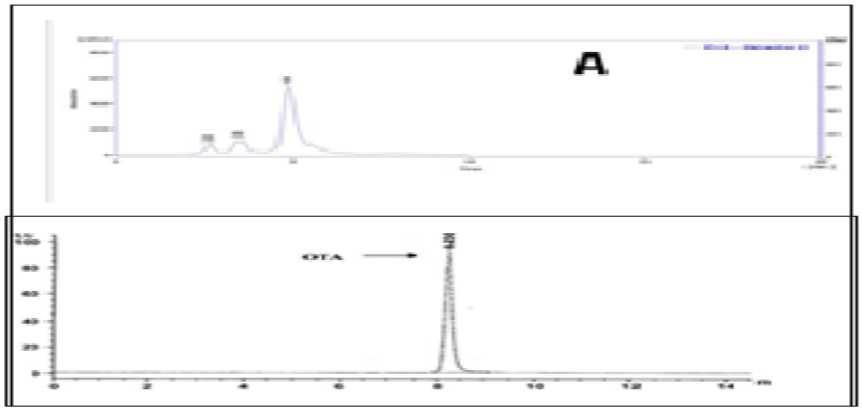


Figure4-8: HPLC chromatogram of Ochratoxin A in Iraqi raisin contaminated with *A. ochraceus*

Table(4-11): HPLC chromatogram of Ochratoxin A in Iraqi raisin contaminated with *A. ochraceus*

Reten. Time	Area	Height	Area	Height	W05
[min]	[mAU.s]	[mAU]	[%]	[%]	[min]
2.583	93.029	12.798	10.4	16.2	0.13
3.443	182.933	18.049	20.5	22.9	0.18
4.860	617.765	48.139	69.1	60.9	0.22
Total	893.727	78.985	100.0	100.0	



Figure(4-9): HPLC chromatogram of Ochratoxin A in Fig fruits contaminated with *A. niger*

Table(4-12): HPLC chromatogram of Ochratoxin A in Fig fruits contaminated with *A. niger*

Reten. Time	Area	Height	Area	Height	W05
[min]	[mAU.s]	[mAU]	[%]	[%]	[min]
2.583	93.029	12.798	10.4	16.2	0.13
3.443	182.933	18.049	20.5	22.9	0.18
4.860	617.765	48.139	69.1	60.9	0.22
Total	893.727	78.985	100.0	100.0	

Table (4-13): HPLC chromatogram of Ochratoxin A in Apricot fruits contaminated with *A. niger*

Reten. Time	Area	Height	Area	Height	W05
[min]	[mAU.s]	[mAU]	[%]	[%]	[min]
2.597	305.873	32.834	5.4	9.3	0.16
3.460	407.810	32.055	7.2	9.1	0.22
4.863	4966.510	286.382	87.4	81.5	0.29
Total	5680.194	351.271	100.0	100.0	

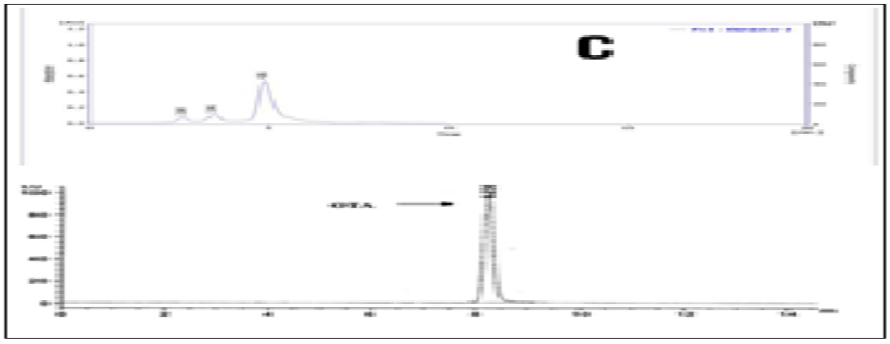
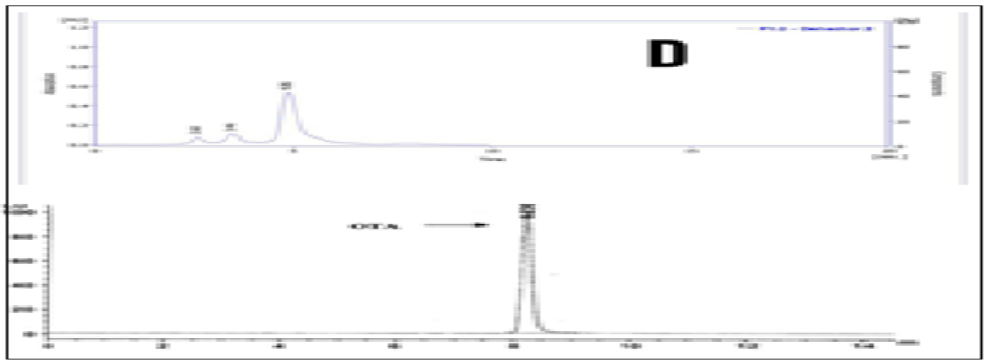


Figure (4-10): HPLC chromatogram of Ochratoxin A in Walnut fruits contaminated with *A. niger*

Table(4-14): HPLC chromatogram of Ochratoxin A in Walnut fruits contaminated with *A. niger*

Reten. Time	Area	Height	Area	Height	W05
[min]	[mAU.s]	[mAU]	[%]	[%]	[min]
2.583	93.029	12.798	10.4	16.2	0.13
3.443	182.933	18.049	20.5	22.9	0.18
4.860	617.765	48.139	69.1	60.9	0.22
Total	893.727	78.985	100.0	100.0	



Figure(4-11): HPLC chromatogram of Ochratoxin A in Prunus fruits contaminated with *A. niger*

Table(4-15):HPLC chromatogram of Ochratoxin A in Prunus fruits contaminated with *A. niger*

Reten. Time	Area	Height	Area	Height	W05
[min]	[mAU.s]	[mAU]	[%]	[%]	[min]
2.597	147.966	19.781	10.0	12.7	0.12
3.460	95.845	12.182	6.4	7.9	0.14
4.863	1242.453	123.202	83.6	79.4	0.18
Total	1486.264	155.164	100.0	100.0	

Table(4_16): Antagonistic activity of *P. ostreatus* and *T. harzianum* against *A. niger* and *A. ochraceus*

4-7 Dual plate assay

4-7-1: Antagonistic activity of *P. ostreatus*

Mycelial growth of *A. niger* and *A. ochraceus*isolates treated with *P. ostreatus* were substantial reducedwhich mostly covered the Petri plates after seven days (Table 4-13) for both pathogenic fungi respectively. This antagonistic fungus grew fairly quickly and was substantial inhibitory effect on growth of both isolates.

The growth of *A. niger* and *A. ochraceus* after two days of inoculation was 2.07 cm and 3.13 cm compared with untreated (3.23 and 4.33 cm) for *A. niger* and *A. ochraceus*respectively. After 4 days of inoculation the dial growth of pathogenic fungi was 3.13 and 4.33 cm compared with untreated pathogenic fungi (4.80 and 5.23 cm) respectively.

In contrast, the growth of pathogenic fungi after 6 days of inoculation was 4.63 and 6.10 cm compared with untreated fungi (5.80 and 7.07 cm) respectively. Differences for treatments, Time and interaction were significant ($P < 0.05$).

Table (4-17): Antagonistic activity of *P. ostreatus* (dual culture), on growth of *A. niger and A. ochraceus* on PDA at 25±1^{o}C after 7 days of inoculation. The data are shown as the average of three replicates with standard error (SE). LSD $_{0.05}$= for treatment=0.645, for day=0.567 and for treatment*day =1.134

Treatments	2 days	4 days	6 days
	Mean±S.E		
***P**.ostreatus *A.niger*	2.07±0.29	3.13±0.35	4.63±0.27
A-niger	3.23±0.33	4.80±0.52	5.80±0.87
P.*ostreatus * A. ochraceus*	3.13±0.24	4.33±0.24	6.10±0.20
A. ochraceus	4.33±0.63	5.23±0.53	7.07±0.59

4-7-2:Antagonistic activity of *T. harzianum*

Similar tendency was occurred with antagonistic activity of *T. harzianum*against mycelial growth of *A. niger and A. ochraceus* (Table 4-14), which substantialreducedafter 2 days of inoculation to 3.10 and 3.17 cm compared to that in control 4.36 and 4.20 cm respectively. While the reduction in mycelial growth of both pathogenic fungi was significantly ($P < 0.05$) increased after 4 days of inoculation: 4.03 and 4.33 cm compared with untreated one: 6,47 and 6.07 cm respectively. In contrast, growth of *A. niger* and *A. Ochraceus* was also decreased significantly after 6 days of inoculation to 5.27 and 5.33 cm compared to the control (7.50 and 6.73 cm). Differences for treatments, Time and interaction were significant ($P < 0.05$).

Table(4_18): Antagonistic activity of *T.harzianum*(dual culture), on growth of *A. niger and A. ochraceus* on PDA at 25±1°C after 7 days of inoculation. The data are shown as the average of three replicates with standard error (SE). LSD $_{0.05}$= for treatment=0.452, for day=0.391 and for treatment*day =0.783

Treatments	**2 days**	**4 days**	**6 days**
	Mean±S.E		
T. *harzianum* **A.niger*	3.10±0.20	4.03±0.08	5.27±0.49
A-niger	4.63±0.08	6.47±0.24	7.50±0.28
T. *harzianum** *A. ochraceus*	3.17±0.20	4.33±0.24	5.33±0.37
*A. ochraceus***l**	4.20±0.23	6.07±0.58	6.73±0.43

4-8: Effect of volatile substances from *T. harzianum*and and *P. ostreatus* against *A. niger and A. ochraceus*

P. ostreatus

The pathogens *A. niger and A. ochraceus*, which were not treated with bioagent fungi, grew so quickly that they covered whole plates in control cultures in seven days, whereas pathogenic fungi that came into contact with volatile compounds from antagonistic fungus*P. ostreatus* had their growth restricted with substantial morphological changes (Table4-14).

The volatile of *P. ostreatus* was considerably reduced (P 0.05) growth diameter of pathogenic fungi after 2 days:*A. niger* (1.83 cm), *A. ochraceus*(2.67cm), 4 days: 3.03, 3.70 cm and after 6 days: 3.43 and 5.93cm respectively compared with control treatment: 2(4.3, 3.97 cm), 4 (4.50 and 4.93) and 6: (5.40 and 6.10 respectively. As the volatile metabolites had a substantial inhibitory effect ($P < 0.05$) on growth of the

pathogenic fungi studied. In contrast, the growth average of both pathogenic fungi was not differed significantly. Differences for treatments, Time and interaction were significant ($P < 0.05$).

Table (4-19): Effect of volatile substances of *P. ostreatus* on growth of *A. niger and A. ochraceus* on PDA at 25±1°C after 7 days of inoculation. The data are shown as the average of three replicates with standard error (SE). LSD $_{0.05}$= for treatment=0.976, for day=0.846 and for treatment*day =1.691

Treatments	2 days	4 days	6 days
	Mean±S.E		
P.*ostreatus**A.niger	1.83±0.41	3.03±0.08	3.43±0.80
A-niger	4.30±0.70	4.50±0.46	5.40±0.20
***P** ostreatus *A. ochraceus*	2.67±0.38	3.70±0.55	5.93±0.34
A. ochraceus	3.97±0.29	4.93±1.03	6.10±0.97

T. harzianum

Pathogenic fungi (*A. niger* and *A. ochraceus*) that came into contact with volatile substances from antagonistic fungus *T. harzianum* were grown fairly slowly with substantial morphological changes, compared with untreated fungi which both of them covered the whole plates in seven days, whereas the growth of bioagent fungus (*T. harzianum*) grew quickly and covered whole plates in the same period (Table 4-16).

The volatile of *T. harzianum*was considerably (P 0.05) reduced growth diameter of pathogenic fungi after 2 days: *A. niger* (3.27 cm), *A. ochraceus* (3.10 cm), 4 days: 4.30, 3.87 cm and after 6 days: 5.67 and 5.00 cm respectively compared with control treatment: 2(5.10 and 6.07 cm), 4 (6.57 and 4.93) and 6: (5.40 and 6.10 respectively. As the volatile metabolites had a substantial inhibitory effect ($P < 0.05$) on growth of the pathogenic fungi studied. In contrast, the growth average of both

pathogenic fungi was not differed significantly after 4 and 6 days of inoculation. Differences for treatments, Time and interaction were significant ($P < 0.05$).

Table (4-20): Effect of volatile substances of *T. harzianum* on growth of *A. niger and A. ochraceus* on PDA at 25±1^{o}C after 7 days of inoculation. The data are shown as the average of three replicates with standard error (SE). LSD $_{0.05}$= for treatment=0.457, for day=0.395and for treatment*day =0.791

Treatments	**2 days**	**4 days**	**6 days**
	Mean±S.E		
*T. harzianum *A.niger*	3.27±0.17	4.30±0.18	5.67±0.44
A-niger	5.10±0.55	6.57±0.31	7.77±0.14
*T. harzianum * A. ochraceus*	3.10±0.15	3.87±0.24	5.00±0.11
A. ochraceus	6.07±0.34	6.57±0.53	7.60±0.30

4-9:Antagonistic effect non-volatile substances (filtrates) of *P. ostreatus* and *T. harzianum*

P. ostreatus

The filtrate of *P. ostreatus*was greatly affected the morphological characteristics as the color, texture and nature of colonies for both *A. niger* and *A. ochraceus* (table 4-17). Filtrate of *P. ostreatus* inhibited the growth of the pathogenic fungi and maximumrestriction was detected and more obviously in *A. niger* followed by *A. ochraceus*. The growth in control samples, on the other hand, mainly covered the entire plate after 7 days of inoculation.

*P. ostreatus*filtrate was considerably (P 0.05) minimized the growth diameter of pathogenic fungi after 2 days: *A. niger* (1.40 cm),

A. ochraceus (1.60 cm), 4 days: 1.73 and 2.37cm) and after 6 days: 3.03 and 2.47 cm respectively compared with control treatment: 2 (3.40 and 4.17 cm), 4 (4.93 and 6.23) and 6: (6.90 and 7.60) respectively. As the metabolites of bioagent fungus had a substantial inhibitory effect ($P < 0.05$) on growth of the pathogenic fungi studied. In contrast, the growth average of both pathogenic fungi was differed significantly after 2, 4 and 6 days of inoculation. Differences for treatments, Time and interaction were significant ($P < 0.05$).

Table(4-21): Effect of nonvolatile substances (filtrate of *P. ostreatus*on growth of *A. niger and A. ochraceus* on PDA at 25±1^{o}C after 7 days of inoculation. The data are shown as the average of three replicates with standard error (SE). LSD $_{0.05}$= for treatment=0.585, for day=0.507and for treatment*day =1.014

Treatments	2 days	4 days	6 days
	Mean±S.E		
***P** ostreatus* *A.niger	1.40±0.30	1.73±0.29	3.03±0.26
A-niger	3.40±0.40	4.93±0.52	6.90±0.20
***P**. ostreatus *A. ochraceus*	1.60±0.30	2.37±0.34	2.47±0.49
A. ochraceus	4.17±0.72	6.23±0.37	7.60±0.51

T. harzianum

The results of this experiment (Fig. 4 -A2 to D2) revealed that the filtrate of *T. harzianum* completely reduced the colony growth of all Fusarium spp. Despite the purity of the filtration procedure to *T. harzianum* fungal filtrate, many spores passed through filtration, and that increased the antagonistic capacities to cover the entire plate and prevent pathogen growth. Therefore, highly inhibitory effect to the fungal pathodens was occurred due to the filtrate and to the direct growth of *T.*

harzianum spores which totally overcome the colony development of fusarium species.

In contrast, the growth of all Fusarium species (*F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2 and *F. oxysporum* AJA) in untreated Petri plates covered the entire plates during the same period.

Table(4-22): Effect of nonvolatile substances (filtrate) of *T. harzianum* on growth of*A. niger and A. ochraceus* on PDA at 25±1^{o}C after 7 days of inoculation. The data are shown as the average of three replicates with standard error (SE). LSD $_{0.05}$= for treatment=0.419, for day=0.362 and for treatment*day =0.725

Treatments	**2 days**	**4 days**	**6 days**
	Mean±S.E		
*T. harzianum *A.niger*	3.07±0.17	3.60±0.30	4.77±0.14
A-niger	4.50±0.28	6.40±0.20	7.43±0.47
*T. harzianum * A. ochraceus*	3.50±0.28	4.60±0.35	5.50±0.28
A. ochraceus	5.10±0.20	6.40±0.40	7.60±0.30

4-10: Antagonist activity of *P. ostreatus* on percentage inhibition of *A. niger and A. ochraceus*

The inhibition percentage for *A. niger and A. ochraceus* mycelia by *P. ostreatus* mycelia was clearly observed using dual culture tests (Table 4-18). Current results revealed that there was substantial effect of *P. ostreatus* against pathogenic fungi.

4-11:Inhibitory effect of *P. ostreatus* on *A. niger*

The antagonist activity of *P. ostreatus*(dual culture) revealed that the percent of inhibition against in growth of *A. niger*(Table 4-18) after 2, 4

and 6 days of inoculation was: 17.6%, 33.5% and 55.1% respectively. But when using the volatile substances, the percent of inhibition to the growth of fungal pathogen after 2, 4 and 6 days of inoculation was: 30.7, 36.5 and 52.4% respectively. In contrast, the percent of inhibition to the growth of *A. niger* treatedwith fungal filtrates of *P. ostreatus* after 2, 4 and 6 days of inoculation was: 41.6%, 55.6 % and 64.9 % respectively. Non-volatile substance (fungal filtrate) was the greatest inhibitory effect on growth of *A. niger* along the period of experiment compared with that of dual culture and volatile substances.

4-12:Inhibitory effect of *P. ostreatus* on *A. ochraceus*

The inhibitory effect of *P. ostreatus* (dual culture) against the growth of *A. ochraceus* (Table 4-18)after 2, 4 and 6 days of inoculation using dual culture technique was: 12.9%, 17.2% and 24.41% respectively. But when using the volatile substances, the percent of inhibition to the growth of fungal pathogen after 2, 4 and 6 days of inoculation was: 17.3, 23.5 and 29.3 % respectively. In contrast, the percent of inhibition to the growth of *A. ochraceus,*treated with fungal filtrates of *P. ostreatus* after 2, 4 and 6 days of inoculation was: 57.2%, 61.4% and 66.7% respectively. Non-volatile substance (fungal filtrate) was the greatest inhibitory effect on growth of *A. ochraceus*along the period of experiment compared with that of dual culture and volatile substances.

Table(4-23): Effect of *P. ostreatus*: antagonism (dual culture), volatile and non-volatile metabolites) on growth as inhibition percentage of *A. niger and A. ochraceus* grown on PDA at 25±1°C after 7 days of inoculation. The data are shown as the average of three replicates.

Bioagents	Treatments	Days after inoculation			Mean
		2	4	6	
A. Niger	Dual culture	17.6	33.5	55.1	35.4
	Volatile metabolites	30.7	36.5	52.4	39.8
	Non-volatile	41.6	55.6	64.9	54
A. ochraceus	Dual culture	12.9	17.2	24.4	18.1
	Volatile metabolites	17.3	23.5	29.3	23.3
	Non-volatile	57.2	61.4	66.7	175.3

4-13:Antagonist activity of *T. harzianum* on percentage inhibition of *A. niger and A. ochraceus*

Using dual culture experiments, the inhibition percentage of*A. niger* and *A. ochraceus* mycelia by *T. harzianum* mycelia was clearly detected (Table 4-19). *T. harzianum* has a significant effect against pathogenic fungus, according to recent findings.

4-13-1 Inhibitory effect of *T. harzianum* on *A. niger*

The antagonist activity of *T. harzianum* (dual culture) demonstrated that at 2, 4, and 6 days after inoculation, the percent of inhibition against the growth of *A. niger* was 30, 32.8, and 37.4 %, respectively. However, after 2, 4, and 6 days of inoculation, the percentage of inhibition to growth of pathogen was 26.9%, 34.9%, and 34.9 %, respectively, when volatile chemicals were used. The suppression to *A. niger* growth treated with filtrate of *T. harzianum* after 2, 4, and 6 days of inoculation was: 31.1, 35.2, and 43.4% respectively. In comparison to dual culture and

volatile chemicals, non-volatile material (fungal filtrate) had the highest inhibitory effect on A. niger growth during the course of the experiment.

4-13-2:Inhibitory effect of *T. harzianum on A. ochraceus*

T. harzianum (dual culture) has an inhibiting effect on *A. ochraceus* growth. The percentage of inhibition to the growth of this pathogen (Table 4-19) after 2, 4 and 6 days of inoculation using dual culture procedure was: 20.7%, 23.5% and 27.5% respectively. However, when using volatile substances, the inhibition percentage to *A. ochraceus*after 2, 4 and 6 days of inoculation was: **37.2**, 39.7and **48.5**% respectively.

After 2, 4, and 6 days of inoculation, the percent inhibition of *A. ochraceus* growth treated with fungal filtrates of *P. ostreatus* was 27.6%, 30.5 and 31.2% respectively. In comparison to dual culture and volatile substances, Volatile substance had the largest inhibitory effect on *A. ochraceus* growth over the course of the experiment.

Table(4-24): Effect of *T, harzianum*: antagonism (dual culture), volatile and non-volatile metabolites) on growth as inhibition percentage of *A. niger and A. ochraceus* grown on PDA at 25±1^{o}C after 7 days of inoculation. The data are shown as the average of three replicates.

Bioagents	Treatments	Days after inoculation			Mean
		2	4	6	
A. niger	Dual culture	30	32.8	37.4	33.4
	Volatile metabolites	26.9	34	34.9	31.9
	Non-volatile	31.1	35.2	43.4	36.5
A. ochraceus	Dual culture	20.7	23.5	27.5	23.9
	Volatile metabolites	37.2	39.7	48.5	41.8
	Non-volatile	27.6	30.5	31.2	29.7

Discussion

4-14-1 Identification of dried fruit contaminated fungi

Studies have shown that the toxin-producing fungi, especially A*spergillus*, are among the main pollutants in dried fruits, and that the fungi *A. niger* and *A. flavus* are the most frequently isolated species in dried fruits (Benliogl*u et al*, 1993; Aziz and Moussa, 2002; Heperkan, 2006; Zohri and Abde Gawad ,2008). The present study agrees with the results of other studies in the dominance of the genus *Aspergillus* in dried fruit samples (Chebil et al., 2020). The same study also confirmed that it is one of the most common species found in food sources are *A. terreus, A.flavus, A. ochraceus, A. niger .A. parasiticus, A. versicolor* and *A. fumigatus.*

OTA, a mycotoxin with nephrotoxic, immunosuppressive, teratogenic, and carcinogenic characteristics, has been detected in Aspergillus species. These results are identical to those previously mentioned (Sambrook and Russell, 2001). The pcr technique is used to provide a molecular diagnosis after phenotypic and microscopic diagnosis. These results are similar to those obtained by Patio, 2005.

Since it is a modern and developed technology and one of the most sensitive and accurate approaches (Edel, 2000; Moslem *et al., 2010).* The PCR technique developed for identifying A. ochraceus in pure culture was also successful in detecting a 400-bp amplicon.

4-14-2 Ochratoxin A detection

HPLC in a stationary phase and a step gradient polarity system of mobile phase were used to identify adequate OTA (Figure (3) in *A. ochraceus* extracted from raisin sample under chromatographic

circumstances. The minimum retention time appears to have been (4.81). The OTA in the figure, on the other hand, was (4.86). Other studies had similar results (Al-Hazmi, 2010). The standard appearance of retention time min in the Ochratoxin A HPLC chromatogram (0.236) Ochratoxin A is found in raisin according to other research. Ochratoxin A was reported in apricot, raisin, nut sample that contaminated with *A. niger*, under chromatographic conditions (Figure 4-4), appearing the maximum of retention period min (4.86) Using chromatographic circumstances, the result of ochratoxin A in ochratoxin A in the fig sample (Chebil et al., 2020) The HPLC chromatogram of Ochratoxin A for the standard (Fig4) and the positive sample at the maximal concentration (2.887)

Heperkan, D. (2006) reported that all the samples tested of dried fruits were contaminated by ochratoxin A and the concentrations ranged between 50-110 µg/kg of apricots, 60-120 µg/kg of They also found that all samples of raisins were naturally free from mycotoxins. Abdel-Sater and and Saber, 1999, recorded a detectable amount of ochratoxin A in two samples of dates (360-450 µg/kg).

Ochratoxin A is a secondary metabolite generated by numerous Aspergillus and Penicillium species and has been detected in a wide range of cereal grains, coffee beans, cocoa, beer, red wine, and most recently in raisins produced in several countries (Trucksess et al.,1999). The difference in toxin concentration may be attributed to Genetic factors, as it was found that the toxin producing isolates had four genes Responsible for the B1 biosynthesis pathway as there Another gene responsible for regulating the activity of these genes is called Structural gene is (Barnett and Hunter, 1972).

Similar to our findings, it was reported the presence of *ochratoxin A* in dried figs in Turkey (Heperkan, 2006). In USA, (Bayman,et al. 2002), examined 50,000 figs for fungal infections and measured ochratoxin content in figs with visible fungal colonies.

Pooled figs infected with *Aspergillus alliaceus* contained ochratoxin A, figs infected with the *A. ochraceus* group had little or none, and figs infected with *Penicillium* had none. Heperkan, 2006, reported that all the samples tested of dried fruits were contaminated by ochratoxin A and the concentrations ranged between 50-110 µg/kg of apricots, 60-120 µg/kg of They also found that all samples of raisins were naturally free from mycotoxins. Abdel-Sater and and Saber, 1999 recorded a detectable amount of ochratoxin A in two samples of dates (360-450 µg/kg). *Ochratoxin A* is a secondary metabolite generated by numerous Aspergillus and Penicillium species and has been detected in a wide range of cereal grains, coffee beans, cocoa, beer, red wine, and most recently in raisins produced in several countries (Ozay et al., 1995). The difference in toxin concentration may be attributed to Genetic factors, as it was found that the toxin producing isolates had four genes Responsible for the B1 biosynthesis pathway as there Another gene responsible for regulating the activity of these genes is called Structural gene (Barnett and Hunter. 1972).

4-14-3 Antagonistic activity of *P. ostreatus* and *T. harzianum* against *A. niger* and *A. ochraceus*

Through dual-culture competition and the synthesis of volatile and non-volatile compounds, T. harzianum and P. ostreatus were tested in vitro against pathogenic fungus A. niger and A. ochraceus. The following details will be covered in greaterdepth:

4-14-4 Antagonistic activity

T. harzianum and P. ostreatus both revealed remarkable antagonistic interactions such as overgrowth and sporulation over *A. niger* and *A. ochraceus* in the current study. They also demonstrated a strong inhibitory impact and showed a reduction in radial growth of pathogenic fungi. Their mycolytic enzyme secretions can be used as a biofertilizer, plant growth promoter, bioremediation, and a boost in agricultural economics (Hyder et al., 2017; Rai et al., 2020).

They also have the potential to be used as a biocontrol agent (*Adedeji et al*., 2016)

T. harzianum and *P. ostreatus* inhibited significantly fungal growth of A. *niger* and A. *ochraceus*, the dual plate culture method showed strong antagonistic action. Overgrowth of both bioagent over the pathogenic fungi was occurred, however only *T. harzianum* sporulated over the pathogenic fungus was reported. The colonies of *A. niger* and *A. ochraceus* treated with *T. harzianum* or *P. ostreatus* were significantly reduced and mostly covered by bioagent fungal mycelia after seven days, as both bioagent fungi developed quickly and were capable of inhibiting *A. niger* and *A. ochraceus* growth. *P. ostreatus* inhibited pathogenic fungi by 17.6 to 55.1 % for *A. niger* and 12.9 to 24.4 % for *A. ochraceus*, while *T. harzianum* inhibited pathogenic fungi by 30 to 37.4 % for *A. niger* and 20.7 to 27.5 % for *A. ochraceus*, indicating that the existence of both antagonistic fungi is necessary to suppress pathogenic fungal mycelia growth (Rahman et al., 2009).

4-14-5 Nonvolatile substances

According to Owaid *et al*., 2017, the secondary metabolism of *P. ostreatus* mycelia is vital in suppressing pathogenic fungal development. Because the chemical composition of oyster mushroom broth varied

based on the type of fungal filtrate, *P. salmoneostramineus* filtrate has a higher activity (Parameswari and Chinnaswamy, 2011).

Secondary metabolic products such as polysaccharides, proteins, enzymes, and triterpenoides suppress pathogenic fungus (Akyuz and Kirbag, 2009).

Both *T. harzianum* and *P. ostreatus* fungal filtrates demonstrated a high inhibitory impact on fungal pathogens, as *A. niger* and *A. ochraceus* colony growth was completely reduced by both bioagent filtrates. Pathogenic species (*A. niger* and *A. ochraceus*) grew across the entire Petri plate in untreated Petri plates at the same time.

P. ostreatus filtrate had a significant impact on morphological traits such as color, texture, and nature of colonies. However, the effect differed depending on the type of bioagent and the type of pathogenic fungi. This pigmentation was caused by mycelial phenoloxidase or peroxidase activity, and the changes in colony features could be attributable to diffusion of metabolites from bioagent fungi (Chaudhary, 2016).

Antagonistic materials produced by microbial metabolites, lytic enzymes, volatile chemicals, as well as other inhibitory substances that are specific or non-specific in nature (Sharma et al., 2013).

Trichoderma spp. synthesize antibiotics with antifungal qualities, such as trichodermin, trichodermol, and herzianolide, so according Küçük and Kivanç (2005).

4-14-6 Volatile substances

T. harzianum and *P. ostreatus* volatile substances showed varied inhibitory effects on *Fusarium* species; both produced poisonous volatiles that had a considerable impact on the pathogens' radial growth, but in

various degrees, which is largely tied to the generation of volatile organic molecules (Azevedo *et al.,* 2020).

They were secreted metabolites that inhibited a variety of *fusarium* species, and some of these metabolites are volatile (Zeilinger *et al*., 2016). (Meena *et al*., 2017). *Fusarium* oxysporum, *Rhizoctonia solani*, *Sclerotium rolfsii, Sclerotinia sclerotiorum,* and *Alternaria brassicicola* growth were dramatically reduced in earlier experiments using bioagent fungi (Amin *et al*., 2010; Meena *et al*., 2017).

Trichoderma produces a number of volatile secondary metabolites, including ethylene, aldehydes, and ketones, all of which are crucial in the prevention of plant disease (Amin *et al*., 2010).

Six *Trichoderma* spp. isolates exhibit volatile potential against seven distinct fungal plant diseases, according to Bhagat *et al*.(2014) . Other studies have revealed the creation of volatile compounds such as acetaldehyde, ethylene, acetone, and carbon dioxide, as well as antibiotics such as *Trichodermin, gliotoxin, viridian,* and *ergokonin* (Siddiquee , 2014; Sharma *et al*., 2016; Rai et al., 2016).

Conclusions

1- The appearance of *Aspergillus* spp more than the rest of the types of fungi.

2-The frequency and appearance of fungi was more in the raisins, Iraqi raisins and apricots than in the rest of the dried fruits.

3- TLC technique demonstrated the ability of *A.ochracuse i*solates.

4- PCR is a highly efficient method for molecular diagnosis of the gene responsible for producing a*chratoxin* toxin in fungal isolates.

5-PCR product analysis of 18S rRNA gene ITS2 gene from extracted DNA of *Aspergillus ochraceus* isolates. Where M: marker (1500-100bp) and the positive *Aspergillus ochraceus* isolates were showed at (420bp) PCR product.

6- The literature on the activity of T. harzianum and P. ostreatus in suppressing dried fruits contaminated fungi like of *A. niger* and *A. ochraceus* is inadequate.

7- This research found that beneficial fungi can reduce OTA levels in .dried fruits by inhibiting the growth of contaminated fung .

Recommendations

1- Quality control of foodstuffs imported from outside Iraq and setting laws on the local manufacturing process to reduce contamination with Ochratoxin A toxins.
2- Using other natural materials to reduce the toxic effects of mycotoxin
3- Control of food storage conditions to prevent the growth of pathogenic fungi.
4- Detection of other types of toxins produced by fungi found in foodstuffs
5- More DNA sequence analysis of different key genes is recommended for more precise species determination
6- Using beneficial fungi *Pleurotus ostreatus* and *Trichoderma harzianum* to control fungi

References

Abarca ML, Accensi F, Bragulat MR,*et al*(2003). Aspergillus carbonariusas the major source of ochratoxin A contamination in driedvine fruits from the Spanish market. J Food Prot; 66:504–506 .

Abarca ML, Bragulat MR, Castella G, Cabanes FJ(1994). Ochratoxin A production by strains of *Aspergillus niger* var. mger Appl Environ Microbiol, 60: 2650—2

Abarca, M. L.; Accensi, F.; Cano, J. and Cabañes, F. J. (2004). Taxonomy and significance of black aspergilli. Antonie van Leeuwenhoek. 86: 33-49.

Abd El-Hai, K. M., & Ali, A. A. (2019). Research Article Formulation of Trichoderma , Saccharomyces and Rhizobium Metabolites Against Damping - off and Root Rot Pathogens in Peanut Plant.

Abdel-Sater, M.A. and Saber, S. M. (1999). Mycoflora and Mycotoxins of some Egyptian dried fruits. Bull. Fac. Sci. Assiut. 28(1-D):91-107.

Accensi F, Cano J, Figuera L,*et al*(1999*)*.New PCR method to differ-entiate species in theAspergillus nigeraggregate. FEMS Mi-crobiol Let;180:191–196 .

Adedeji KO, Aduramigba MO (2016). In vitro evaluation of spent mushroom compost on growth of *Fusarium oxysporium* f. sp. lycopersici. Advances in Plants and Agriculture Research.;4(4):332-9.

Kayas , M., & Kirbag, S. (2009). Antimicrobial activity of Pleurotus eryngii var. ferulae grown on various agro-wastes. *EurAsian Journal of BioSciences*, *3*(1), 58-63.

Al-Ameri, H., A Dawood, Z., & N Mula Abed, F. (2018). Isolation and Identification of Fungi From Strawberry Seedlings and its Biological Agent and Fungicides. Rafidain Journal of Science, 27(1), 82-94 .

Alexopoulos CJ, Mims CW , Blackwell M (1996). Introductory Mycology. John W iley and Sons. ISBN 0471522295, p. 17.

Al-Hazmi, N. A. (2010). Determination of patulin and ochratoxin A using HPLC in samples in Saudi Arabia. Saudi journal of biological sciences, 17(4), 353-359.

Alhussaini, M. S. (2012). Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from Saudi Arabia. Journal of American Science; 8(12):525-534.

Al-mohaidi, M., & Saadon, A. S. (2020). Inhibition growth of Aspergillus niger that isolated from some fodder grains by using the Pleurotus ostreatus and Calcium citrate. *Al-Qadisiyah Journal of Pure Science*, *25*(3), 18-29.

Amezqueta, S., Schorr-Galindo, S., Murillo-Arbizu, M., Gonzalez-Peñas, E., De Cerain, A. L., & Guiraud, J. P. (2012). OTA-producing fungi in foodstuffs: A review. Food Control, 26(2), 259-268

Amin F, Razdan VK, Mohiddin FA, Bhat KA, Sheikh PA(2010). Effect of volatile metabolites of Trichoderma species against seven fungal plant pathogens in-vitro. Journal of Phytology. Dec 19;2(10).

Attanayake, R.N.; Glawe, D.A.; Dugan, F.M.; Chen, W. (2009). Erysiphe trifolii causing .

Azevedo DM, da Silva Rocha F, Fernandes MD, da Costa CA, Muniz MD, Barroso PD, Amaral FL, do Rosário Barbosa DM(2020). Antagonistic effect of Trichoderma isolates and its metabolites against Fusarium solani and F. oxysporum in chickpea. Brazilian Journal of Development. Jun 10;6(6):36344-61.

Aziz, N. H. and Moussa, L. A. A.(2002). Influence gamma radiation on mycotoxin producing moulds and mycotoxins in fruits. Food Control, 13: 281-288.

Aziz, N. H. and Moussa, L. A. A.(2002). Influence gamma radiation on mycotoxin producing moulds and mycotoxins in fruits. Food
. Control, 13: 281-288

Barkai-Golan, R. (2002). An Annotated Check -list of Postharvest Fungal Diseases of Fruits and Vegetables in Israel, Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Volcani Center.

Barnett, H.L. and B.B. Hunter.(1972). Illustrated genera of imperfect fungi, 3rd ed. Burgess Publishing Company, USA. 241 pp.

Basappa, S.S., Shantha, T., (1996). Methods for detoxification of aflatoxins in foods and feeds – a critical appraisal. J. Food. Sci. Technol. Ind. 33, 95–107.

Battilani P, Barbano C, and Logrieco A (2008). Risk assessment andsafety evaluation of mycotoxins. In:Mycotoxins in Fruits andVegetables. Barkai-Golan R and Paster N (eds.). The Nether-lands: Academic Press,

Battilani P, Magan N, Logrieco A.(2006). European research on ochratoxin A in grapes and wine. Int J Food Microbiol. 111: S2–S4

Bayman, P., Baker, J. L., Doster, M. A., Michailides, T. J., & Mahoney, N. E. (2002). Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*. Applied and environmental microbiology

Bayman, P., Baker, J. L., Doster, M. A., Michailides, T. J., & Mahoney, N. E. (2002). Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. Applied and environmental microbiology.

Benlioglu S., A. Yildiz, and N. Baspinar.(2008). Aydin ili'nden ihraç edilenkuru incirlerde fungal bulasiklik. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, 5: 3-8.

Bennett J.W., Klich M(2003). Mycotoxins. Clin. Microbiol. Rev. 2003;16:497–516. doi: 10.1128/CMR.16.3.497-516 .

Bertagne, S.; Casta, J. M. (2006). Towards anucleic acid-based diagnosis in clinical prasitology and mycology. Clinica chimica acta, 363:221-228.

Bhagat S, Birah A, Kumar R, Yadav MS(2014). Chattopadhyay C. Plant disease management: prospects of pesticides of plant origin. InAdvances in Plant Biopesticides (pp. 119-129). Springer, New Delhi.

Bhat, A.R.; Bhat, G.V.; Shenoy G.G. (2010).Synthesis and in vitro antimicrobial activity of new 1,2,4-Triazoles. Journal of Pharmacy and Pharmacolog, 53(2):267-272.

Bluhm BH, Flaherty JE, Cousin MA,*et al*(2002).PCR detection of fumonisin and trichothecence producingFusariumspecies. JFood Prot;65:1955–1961 .

Botes M, de Kwaadsteniet M, Cloete TE(2011). Application of quantitative PCR for the detection of microorganisms in water. Food Microbiol. 28(5):848–61.

Bretholtz-Emanuelsson A, Olsen M(1993). Oskarsson A, Palminger I, Hult K Ochratoxin A in cow'smilk and human milk with corresponding human blood samples. / AOAC Int; 76 842-6

Bryden, W. L. (2007). Mycotoxins in the food chain: human health implications. Asia Pacific J. Clin. Nutrition, 16(S1): 95-101.

Campos-Avelar, I., Colas De La Noue, A., Durand, N., Fay, B., Martinez, V., Fontana, A., ... & Schorr-Galindo, S. (2020). Minimizing Ochratoxin A Contamination through the Use of Actinobacteria and Their Active Molecules. *Toxins*, *12*(5), 296.

Castegnaro M, Plestina R, Dirheimer G, Chernozemsky IN, Bartsch H(1991). (Eds) Mycotoxins,Endemic Nephropathy and Urinary Tract Tumours IARC Scientific Publicanons No 115Lyon, France. World Health Organization/International Agency for Research on Cancer,.

Chaudhary R, Tripathi A(2016). Interactions between mushrooms and fungi in dual-culture experiments. Int. J. Adv. Res.–2016.–4. 6:482-93.

Chebil, S., Oueslati, S., Ben-Amar, A., & Natskoulis, P. (2020). fungi and Ochratoxin A determination in dried grapes marketed in Tunisia. Annals of Microbiology, 70(1), 1-9.

Chen, W.; Li, C.; Zhang, B.; Zhou, Z.; Shen, Y.; Liao, X.; Yang, J.; Wang, Y.; Li, X.; Li, Y.; et al)(2018). Advancesin Biodetoxification of Ochratoxin A-A Review of the Past Five Decades.Front. Microbiol.,9,.[CrossRef]

Chin, J.P.; Megaw, J.; Magill, C.L.; Nowotarski, K.; Williams, J. P.; Bhaganna, P.; Linton, M.; Patterson, M. F.; Underwood, G. J. C.; Mswaka, A. Y.; Hallsworth, J. E. (2010) Solutes determine the temperature windows for microbial survival and growth. Proceedings of the National Academy of Sciences of the United States of America, 107 (17): 7835-7840.

Cole RJ, Cox RH (1981). Handbook of toxic fungal metabolites. Academic Press, New York, N.Y .

Conkova, E.; Laciakova, A. N. N. A.; Styriak, I.; Czerwiecki, L. U. D. W. I. K. and Wilczinska, G. (2006). Fungal contamination and the levels of mycotoxins (DON and OTA) in cereal samples from Poland and East Slovakia. Czech J. Food Sci., 24(1): 33-40.

Da RR, Palacios V, Combina M,*et al*(2002).Potential ochratoxin Aproducers from wine grapes in Argentina and Brazil. FoodAddit Contam;19:408–414

Dachery, B., Hernandes, K. C., Veras, F. F., Schmidt, L., Augusti, P. R., Manfroi, V., ... & Welke, J. E. (2019). Effect of Aspergillus carbonarius on ochratoxin a levels, volatile profile and antioxidant activity of the grapes and respective wines. Food Research International, 126, 108687.

Edel,V.(2000). Polymerase chain reaction in mycology an overview in applications of PCR in mycology, In:Bridge ,P.D. ; Arora ,D.K. ;Reddy C.A. andElander ,R.P.(eds),CAB. Internationl publishing.

Heperkan, D. (2006). The importance of mycotoxins and a brief history of mycotoxin studies in Turkey. ARI Bulletin of Istanbul Technical University, 54: 18-27.

Dachery, B., Hernandes, K. C., Veras, F. F., Schmidt, L., Augusti, P. R., Manfroi, V., ... & Welke, J. E. (2019). Effect of Aspergillus carbonarius on ochratoxin a levels, volatile profile and antioxidant activity of the grapes and respective wines. Food Research International, 126, 108687.

Deng ZL, Ma Y (1998). Aflatoxin sufferer and P53 gene mutation in hepatocellular carcinoma., W orld J. Gastroenterol., 4: 28.

Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, *57*(1), 25-IN3.

Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of Trichoderma: II. Production of volatile antibiotics. Transactions of the British Mycological Society, 57(1), 41-IN4.

Doohan FM, Weston G, Rezanoor HN, Parry DW, Nicholson P (1999).Development and use of a reverse transcription-PCR assay tostudy expression of tri5 byFusariumspecies in vitro and inplanta. Appl Environ Microbiol 65:3850–3854

Doshi, P., P. Adsule, and K. Banerjee. (2006). Phenolic composition and antioxidant activity in grapevine parts and berries

(*Vitis vinifera L.*) cv Kishmish Chornyi (Sharad Seedless) during maturation. Int. J. Food Sci. Technol. 41: 1–9.

Doster, M. A. and Michailides, T. J. (1998). Production of bright greenish yellow fluorescence in figs infected by Aspergillus species in California orchards. Plant Dis., 82(6): 669-673.

Eaton, D. L. and Groopman, J. D. (1994). The toxicology of aflatoxins: human health, veterinary, and agricultural significance. Academic Press., New York.

Edel,V.(2000). Polymerase chain reaction in mycology an overview in applications of PCR in mycology ,In:Bridge ,P.D. ; Arora ,D.K. ;Reddy C.A. andElander ,R.P.(eds),CAB.

Efuntoye, M. O. (1996). Fungi associated with Internationl publishing. herbal drug plants during storage. Mycopathologia, 136(2): 115-118.

Ehrlich KC, Mantalbano BG, Cotty PJ (2005). Divergent regulation of aflatoxin production at acidic pH by two *Aspergillus* strains.Mycopathol 159:579–581

Elad, Y. (2000). Biological control of foliar pathogens by means of Trichoderma.harzianum and potential modes of action. *Crop protection*, *19*(8-10), 709-714.

Elad, Y., Chet, I., Boyle, P., & Henis, Y. (1983). Parasitism of Trichoderma spp. on Rhizoctonia solani and Sclerotium rolfsii-scanning electronmicroscopy and fluorescence microscopy. *Phytopathology*, *73*(1), 85-88.

Ellner FM (2005). Results of long-term field studies into the effect ofstrobilurin containing fungicides on the production of mycotox-ins in several winter wheat varieties. Mycol Res 21:112–115.

Embaby, E. M.; Hagagg, L. F. and Abdel-Galil, M. M. (2012). Decay of some fresh and dry fruit quality contaminated by some mold fungi. J. Appl. Sci. Res., 8(6): 3083-3091.

Espinosa Calderón, A., Tadeo Robledo, M., Gómez Montiel, N., Sierra Macías, M., Virgen Vargas, J., Palafox Caballero, A., ... & González Rojo, I. (2011). 'V-55 A', variedad de maíz de grano amarillo para los Valles Altos de México. Revista fitotecnia mexicana, 34(2), 149-150 ..

Essono, G., Ayodele, M., Akoa, A., Foko, J., Filtenborg, O., & Olembo, S. (2009). Aflatoxin-producing Aspergillus spp. and aflatoxin levels in stored cassava chips as affected by processing practices. Food Control, 20(7), 648-654.

EUeno Y. (Ed) (1983). Tnchothecenes - Chemical, Biological and Toxicological Aspects. Amsterdam:Elsevier,

FAO. (1998). Mycotoxin Prevention and Control in Food Grain. Food and Agricultural Organization of the United Nations. Rome.

Farjood, E. and Banihashemi, Z. (2013). Incidence of Aspergillus Species and Mycotoxins In Dried Figs In Southern Iran. Iran. J. Plant Path., 48(4): 183-189.

Fungaro MHP, Vissotto PC, Sartori D,*et al* (2004).A molecular methodfor detection ofAspergillus carbonariusin coffee beans. CurrMicrobiol;49:123–127.

Geisen, R. (2007). Molecular detection and monitoring of fungi. In: Food Mycology: a Multifaceted Approach to Fungi and Food (J. Dijksterhuis, R. A. Samson, ed.), CRC Press, Boca Raton, FL, USA, 255-278.

Gilman, S. E., Cochran, S. D., Mays, V. M., Hughes, M., Ostrow, D., & Kessler, R. C. (2001). Risk of psychiatric disorders among individuals reporting same-sex sexual partners in the National Comorbidity Survey. American journal of public health, 91(6), 933.

Gregory, P. H. (1973). The microbiology of the atmosphere. (ed. 2) John Wiley & Sons. New York and London.

Guglielmo, F.; Gonthier, P.; Garbelotto, M.; Nicolotti, G. (2008). A PCR- based method for the identification of important wood rotting fungal taxa within Ganoderma, Inonotus s.l. and Phellinus s.l. FEMS Microbiol Lett, 282: 228-237.

Hawksworth DL (2006). The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycology Research. 95: 641–655.

Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P., & Denning, D. W. (2007). Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology, 153(6), 1677-1692.

Heenan CN, Shaw KJ, Pitt Jl (1998). Ochratoxin A production by AspergiUus carbonanus and A.niger isolates and detection using coconut cream agar. / Food Mycol; 1 67-72

Hell, K.; Cardwell, K. F.; Sétamou, M.; Peohling, H. M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. Journal of Stored Product Research. 36:365-382.

Heperkan, D. (2006). The importance of mycotoxins and a brief history of mycotoxin studies in Turkey. ARI Bulletin of Istanbul Technical University, 54: 18-27..

Howell, C. R. (2003). Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. *Plant disease*, *87*(1), 4-10.

Hubbard, J., Harman, G., & Hadar, Y. (1983). Effect of soilborne Pseudomonas spp. on the biological control agent. *Trichoderma hamatum*, 655-659.

Iamanaka, B. T.; Taniwaki, M. H.; Menezes, H. C.; Vicente, E. and Fungaro, M. H. P. (2005). Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. Food Additives and Contaminants, 22(12): 1258-1263.

IARC (1993).International Agency for Research on Cancer. Ochratoxin A. monographs on the evaluation of carcinogenic risks to human: some naturally occurring substances, 489-521.

IARC. (1993). IARC Monographs on the Evaluation of Carcinogenic risks to Humans, and International Agency for Research on Cancer. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins /this publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 9-16

ICMSF. (1996). International Commission on Microbiological Specification for FoodToxigenic Fungi:Aspergillus In ICMSF, Microorganism in Food. Characteristics of Food Pathogens,London, Blackie Academic And Professional, 347-81..

Javanmard, M. (2010). Occurrence of mould counts and Aspergillus species in Iranian dried Figs at different stages of production and processing. J. Agric. Sci. Technol., 12: 331-338.

Kaaya, A. N.; Warren, H.; Adipala, E. (2000). Moulds and aflatoxin contamination of maize and groundnuts in Mayuge and Kumi districts of Uganda. Muarik Bulletin, 3: 33-41.

King ADJR, Hocking AD, and Pitt JI(1979). Dichloran-rose bengalmedium for enumeration andisolation of molds from foods.Appl Environ Microbiol;37:959–964.

Klich MA and Pitt JI.A (1988). Laboratory Guide to Common *Aspergillus* Species and their Teleomorphs. North Ryde, Australia: CSIRODivision of Food Processing,.

Krogh P, Hald B, Englund P, Rutqvist L, Swahn O(1974). Contamination of Swedish cereals withochratoxin A. Ada Pathol Mtcrobiol Scand, Sect B; 82: 301-2.

Kuchari, M. G. M.; Qattan, A. T. M. (2001). Environmental factors affecting growth and Aflatoxin production by locally Aspergillus flavus and Aspergillus parasiticus. (Arab.) Unv. Journal of Agricultural Science., Ain-shams Univ., Cairo, 9(2): 583-593.

Küçük, Ç., & Kivanç, M. (2004). Isolation of Trichoderma spp. and determination of their antifungal, biochemical and physiological features. *Turkish Journal of Biology*, *27*(4), 247-253.

Kuzmanovska, B., Rusevski, R., Jankulovska, M., & Oreshkovikj, K. B. (2018). Antagonistic activity of Trichoderma asperellum and Trichoderma harzianum against genetically diverse Botrytis cinerea isolates. *Chilean journal of agricultural research*, *78*(3), 391-399.

Leong SL, Hocking AD, and Pitt JI(2004) . Occurrence of fruit rot fungi (Aspergillus section Nigri) on some drying varieties of irrigatedgrapes. Austr J Grape Wine Res;10:83–88 .

Licitra, L., Perrone, F., Bossi, P., Suardi, S., Mariani, L., Artusi, R., ... & Pilotti, S. (2006). High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. Journal of Clinical Oncology, 24(36), 5630-5636.

Lieckfeld, E.; Seifer, T. K. A. (2000). An evaluation of the use of ITS sequences in the taxonomy of the Hypocreales. Studies in Mycology, 45: 35-44.

Magnoli C, Astoreca A, Ponsone L,*et al* (2004).Survey of mycofloraand ochratoxin A in dried vine fruits from Argentina markets.Lett Appl Microbiol;39:326–331.

Mehra, S. K.; Jaitly, A. K.and Jaitly (1995). PH and temperature optima for growth and sporolation in city waste.mycosci, 36: 243-246.

Menten, J. O. M., Machado, C. C., Minussi, E., Castro, C., & Kimati, H. (1976). Effect of some fungicides on the growth of Macrophomina phaseolina (Tass.) Goid in vitro. Fitopatologia brasileira.

Milićević, D. R., Škrinjar, M., & Baltić, T. (2010). Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. Toxins, 2(4), 572-592.

Mokhtar, H., & Aid, D. (2013). Contribution in isolation and identification of some pathogenic fungi from wheat seeds, and evaluation of antagonistic capability of Trichoderma harzianum against those isolated fungi in vitro. *Agric. Biol. JN Am*, *4*(2), 145-154.

Moore GS, Jaciow DM (1979). Mycology for the Clinical Laboratory Reston Publishing Company Virginia. pp. 161-198..

Moslem, M. A., Mashraqi, A., Abd-Elsalam, K. A., Bahkali, A. H., & Elnagaer, M. A. (2010). Molecular detection of ochratoxigenic Aspergillus species isolated from coffee beans in Saudi Arabia. Genetics and Molecular Research, 9(4), 2292-2299.

Moss, M.O. (1998). Recent studies of mycotoxins.Journal of AppliedMicrobiology Symposium Supplement84,62S±76S.

Moubasher.,A. A. H.Abdel-Hafez, S. I, & Barakat, A. (1993). Seasonal variationsof fungi of outdoor air and sedimented .dust at Assiut region, Upper Egypt. Grana, 32(2), 115-121

Mueller GM, Schmit JP (2006). Fungal biodiversity: what do we know? What can we predict?. Biodiversity Conservation, 16: 1–5.

Nicolás-Santiago, D.; Regalado-González, C.; García-Almendárez, B.; Fernández, F. J.; Téllez-Jurado, A. and Huerta-Ochoa, S. (2006). Physiological, morphological, and mannanase production studies on Aspergillus niger uam-gs1 mutants. Electronic J. Biotechnol., 9(1).

Niessen L. Molecular detection of ochratoxin A producers: anupdat

Nowshehri, J.A., Z.A. Bhat, M.Y. Shah. (2015). Blessings in disguise: Bio-functional benefits of grape seed extracts. Food Res. Int. 77(3): 333-348. Talbot PHB (1971). Principles of Fungal Taxonomy. Macmillan London, pp. 3-10.

Ozay, G.; Aran, M. and Pala, M. (1995). Influence of harvesting and drying technique on mycoflora and mycotoxins of figs. Die Nahrung, 39(2):156-165.

Ozay, G.; Aran, M. and Pala, M. (1995). Influence of harvesting and drying technique on mycoflora and mycotoxins of figs. Nahrung, 39:156-165. .

Patiño B, González-Salgado A, Gonzalez-Jaen M and Vázquez C (2005). PCR detection assays for the ochratoxin-producing Aspergillus carbonarius and Aspergillus ochraceus species. Int. J. Food Microbiol. .104: 207-214

Payne, G. A. (1998). Process of contamination by aflatoxin-producing fungi and their impact on crops. Mycotoxins in agriculture and food safety, Chapter 9, pp 279-306

Peraica, M., Flajs, D., Domijan, A. M., Ivić, D., & Cvjetković, B. (2010). Ochratoxin A contamination of food from Croatia. Toxins, 2(8), 2098-2105.

Pereira JA (2013). Vitis vinifera leaves towards bioactivity. Industrial Crops and

Péteri, Z.; Téren, J.(2007). Vágvölgyi, C.; Varga, J. Ochratoxin degradation and adsorption caused byastaxanthin-producing yeasts.Food Microbiol.,24, 205–210. [CrossRef

Pezzuto, J.M. (2008). Grapes and human health: A perspective. J. Agric. Food Chem. 56: 6777–6784. Mavor JW , Harold HW (1966). General Biology Six Edition collier- Macmillan London- New York, pp. 4-12.

Pitt JI, Hocking AD (1997). Fungi and Food Spoilage, 2nd edn London Blackie .

Pitt JI, Hocking AD, Bhudhasamai K, Miscamble BF, Wheeler KA, Tanboon-Ek P. (1993). The normalmycoflora of commodities from Thailand. 1. Nuts and oilseeds. Intj Food Microbiol, 20:211-26

Pitt JI, Hocking AD (1998). Miscamble BF et al. The mycoflora of food commodities from Indonesia./ Food Mycol, 1: 41-60

Pitt, J. I. (1994). The current role of Aspergillus and Penicillium in human and animal health. *Journal of medical and veterinary mycology*, *32*(sup1), 17-32.

Pitt, J. I. (1994). The current role of Aspergillus and Penicillium in huma

Pitt, J. I. and Hocking, A. D. (1997). Fungi and food spoilage. 2nd edLondon, Blackie Academic and Professional,.pp.511.

Pitt, J. I.; Hoching, A. D. (1997). Fungi and Food Spoilage.2nd Edn Springer, New York, Isbn-10: 593.

Pfohl-Leszkowicz, A., & Manderville, R. A. (2007). Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Molecular nutrition & food research, 51(1), 61-99.

Ramirez ML, Chulze S, Magan N (2006). Temperature and wateractivity effects on growth and temporal deoxynivalenol produc-tion by two Argentinean strains of Fusarium graminearum onirradiated wheat grain. Int J Food Microbiol 106:291–296

Raper, K. B. and Fennell, D. I. (1965). The genus Aspergillus. The Williams & Wilkins Comp,. Baltimore, USA, pp. 686..

Ribeiro JMM, Cavaglieri LR, Fraga ME, Direito GM, Dalcero AM,Rosa CAR (2006) . Influence of water activity, temperature andtime on mycotoxins production on barley rootlets. Lett ApplMicrobiol 42:179–184

Rodrigues, J. C., Ochoa, R., & Kane, E. C. (2007). First report of Raoiella indica Hirst (Acari: Tenuipalpidae) and its damage to coconut palms in Puerto Rico and Culebra Island. International Journal of Acarology, 33(1), 3-5.

Ruston, I.Y.S.,(1997). Aflatoxin in food and feed: Occurrence, legislation and inactivation by physical methods. Food Chem .

Saadullah, A. A., & Abdullah, S. K. (2018). Fungal contamination of dried vine fruits and ochratoxin a detection in grape juice from Duhok, Iraq. *Revista Innovaciencia*, 6(2), 1-8.

Sambrook, J. and Russell, D. W. (2001). Molecular cloning: a laboratory manual. 3rd ed Gold spring Harbor laboratory. New York. USA . .

Samson, R. A.; Houbraken, J.; Thrane, U.; Frisvad, J. C. and Andersen, B. (2010). Food and indoor fungi. CBS-KNAW Fungal Biodiversity Centre Utrecht.

Sánchez-Hérvas M, Gil JVV, Bisbal F, Rámon D, et al. (2008). Mycobiota and mycotoxin producing fungi from cocoa beans. Int. J. Food Microbiol. 125: 336-340.

Sancho LG, de la Torre R, Horneck G, Ascaso C, de Los Rios A, Pintado A, W ierzchos J, Schuster M (2007). "Lichens survive in space: Results from the 2005 LICHENS experiment". Astrobiology 7(3): 443-454.

Sava, V., Reunova, O., Velasquez, A., Song, S., & Sanchez-Ramos, J. (2006). Neuroanatomical mapping of DNA repair and antioxidative responses in mouse brain: Effects of a single dose of MPTP. Neurotoxicology, 27(6), 1080-1093.

Schmidt-Heydt M, Baxter E, Geisen R, Magan N (2007). Physiolog-ical relationship between food preservatives, environmental

Schmidt-Heydt M, Magan N, Geisen R (2008). Stress inductionofmycotoxin biosynthesis genes by abiotic factors. FEMS Micro-biol Lett 284:142–149

Scott ,PM. ,Pemcillium mycotoxins In Wyllie TD, Morehouse LG (Eds) (1977). Mycotoxic Fungi,Mycotoxins, Mycotoxicoses, an Encyclopedic Handbook. Vol. 1. Mycotoxigemc Fungi. NewYork: Marcel Dekker,; 283-356.

Shetty, P.H.; Jespersen, L(2006). Saccharomyces cerevisiae and lactic acid bacteria as potential mycotoxindecontaminating agents.Trends Food Sci. Technol.,17, 48–55. [CrossRef]

Siameto, E. N., Okoth, S., Amugune, N. O., & Chege, N. C. (2011). Caracterización molecular e identificación de aislamientos de biocontrol de Trichoderma harzianum del Distrito de Embu, Kenia. *Tropical and subtropical agroecosystems*, *13*(1), 81-90.

Skidmore, A. M., & Dickinson, C. H. (1976). Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. *Transactions of the British Mycological Society*, *66*(1), 57-64.

Smith C, Osborn M(2009). Advantages and limitations of quantitative PCR (qPCR)-based approaches in microbial ecology. FEMS Microbiol Ecol.;67(1):6–20 .

Snowdon, A. L. (1990). A Colour atlas of post-harvest diseases and disorders of fruits and vegetables. Volume 1.General Introduction and Fruits. London: Wolfe Scientific Ltd. pp. 302.

Stahlberg A, Thomsen C, Ruff D, *et al* (2012). Quantitative PCR analysis of DNA, RNAs, and Proteins in the same single cell. Clin Chem.; 58:1682–1691.

Tapwal, A., Singh, U., Singh, G., Garg, S., & Kumar, R. (2011). In vitro antagonism of Trichoderma viride against five phytopathogens. *Pest Technol*, *5*(1), 59-62.

Trucksess, M.W.; Giler, J.; Young, K.; White, K.D. and Page, S.W. (1999). Determination and survey of ochratoxin A in wheat, barley and coffee-1997. Journal of AOAC International. 82(1): 85-89..

Valasek MA, Repa JJ(2005). The power of real-time PCR. Advances in physiology education.;29(3):151–9. .

Van der Merwe KJ, Steyn PS, Foune L, Scott DB, Theron JJ(1965). Ochratoxin A, a toxic metaboliteproduced by Aspergillus ochraceus Wilh Nature; 205: 1112 -

Vanhoutte, I.; Audenaert, K.; De Gelder, L. (2016). Biodegradation of mycotoxins: Tales from known and unexploredworlds.Front. Microbiol., 7. [CrossRef] [PubMed]

Varga J, Kevei E, Rinyu E, Teren J, Kozakiewicz Z.(1996). Ochratoxin production by AspergiUusspecies. Appl Environ Mtcrobiol; 62: 4461-4

Vujanovic, V., Smoragiewicz, W., & Krzysztyniak, K. (2001). Airborne fungal ecological niche determination as one of the possibilities for indirect mycotoxin risk assessment in indoor air. Environmental Toxicology: An International Journal, 16(1), 1-8..

Walker, R., Devries, J. W., Trucksess, M. W. & Jackson, L. S. (2002). Risk assessment of ochratoxin: current views of the European scientific committee on food, the JECFA and the codex committee on food additives and contaminants. Adv. Exp. Med. Biol. 504, 249

Wang, Q. H., Kalantar-Zadeh, K., Kis, A., Coleman, J. N., & Strano, M. S. (2012). Electronics and optoelectronics of two-dimensional transition metal dichalcogenides. Nature nanotechnology, 7(11), 699-712.

Welke, J. E. (2019). Fungal and mycotoxin problems in grape juice and wine industries. Current Opinion in Food Science, 29, 7-13.

Welke, J. E. (2019). Fungal and mycotoxin problems in grape juice and wine industries. Current Opinion in Food Science, 29, 7-13.

Wilson, D. M. and Payne, G. A. (1994). Factors affecting *Aspergillus flavus* Group Infection and Aflatoxin contamination of Crops. The Toxicology of Aflatoxins Human Health, Veterinary, and Agricultural Significance.

Xu, Y.; Bianchini, A. and Hanna, M. A. (2011). Evaluation of Mold and Mycotoxin Contaminations in Hybrid Hazelnuts Grown in Nebraska. J. Food Processing and Technology.

Youssuf, G.; Robert, L. M.; Mahendra, R. (2009).Current Advances in Molecular Mycology. Editore,Nova Science Publishers.Inc.New York.

Zohri, A. A.; Abdel-Gawad, K. M. (1993). Survey of microflora and mycotoxins of some dried fruits in Egypt. Journal of Basic Microbiology, 33: 279-288 .

الخلاصه

تعتبر الفواكه المجففة (الزبيب والزبيب العراقي *Vitis Vinifera* L . والتين *Ficus Caric* L. والمشمش *prunus armeniaca* L. والبندق *Juglans regia L* والبرقوق *common plums*) من الأطعمة الصحية وتستخدم في الصناعات الغذائية والمعجنات او الاستخدام المباشر . هدفت الدراسة إلى توصيف Ochratoxin A (OTA) وامكانية السيطره على الملوثات الفطريه المنتجة للأوكراتوكسين باستخدام الفطريات النافعه وتناولت الدراسة التحقق من وجود هذه الفطريات في الثمار المجففة ، والتوصيفات المظهريه لمستعمراتها ، ونسب الظهور والتكرار والتشخيص الجزيئي للفطر *A. A.ochraceus* ودراسة قدراته مع الفطر *A. niger* على إنتاج سم OTA من خلال اعتماد كروماتوغرافيا الطبقة الرقيقة (TLC) و HPLC بالإضافة إلى إمكانيات السيطرة على الملوثات الفطريه باستخدام الفطريات المفيدة. اجمالا، تم الحصول على 380 عزلة فطرية من الفواكه المجففة الأكثر انتشارا في العراق ، مثل الزبيب والتين والمشمش والبندق والبرقوق. باعتماد الخصائص المظهرية للمستعمرات الفطريه مثل لون المستعمرة ، وحوافها ، والملمس ، ولون خلفية المستعمره الفطريه ، شخصت عدة أنواع مختلفة ، تنتمي إلى أربعة أجناس مختلفة هي:

A. niger (80), *A. terrus* (50), *A. flavus* (55), *A. ochraceus* (40). *Fusarium* (66) *Nigrosora* (35) and *Penicillium* (54).

اختلفت نسبة الفطريات الملوثة التي ظهرت على الفواكه المجففة باختلاف نوع الثمار المجففة والفطر الملوث. وظهرت الاصابه على المشمش والزبيب والزبيب العراقي بجميع فطريات التلوث بينما تفاوتت الإصابة بالفواكه المجففة الأخرى (البندق والتين والبرقوق). أظهرت الدراسه أن الفطريات المنتجة للسموم ، وخاصة *Aspergillus* ، هي من بين الملوثات الرئيسية للفواكه المجففة وأن الفطريات *A. niger* و *A. flavus* هما أكثر الأنواع المعزولة في الفواكه المجففة.

شكلت ثمارالمشمش (35٪) وهي الأعلى نسبة للاصابه بالفطريات الملوثة ، يليها الزبيب (32٪) ، الزبيب العراقي (26.2٪) ، البرقوق الشائع (2.6٪) ، التين (2.4٪) ، والبندق (1.6٪). أظهرت نتائج اختبار TLC أن 6 عزلات من *A. ochraceus* أنتجت معظمها OTA في مختلف أنواع الثمار المجففة المدروسة. أكدت النتائج ايضا تحليل منتج PCR للجين 18 S

rRNAمن الجين ITS2 من الحمض النووي المستخرج من عزلات *A. ochraceus* وأظهرت أن عزلات هذا الفطر موجبة عند (420 زوج قاعدي).

بينت النتائج قدرات كل من *Trichoderma harzianum* و *Pleurotus ostreatus* على تثبيط *A. niger* و *A. ochraceus* باستخدام ثلاث طرق: التضاد المباشر ومن خلال تأثير راشح الفطرين او تأثير المواد المتطايرة. كان لكل من العوامل الحيوية تأثير مثبط معنوي على معدل نمو الفطريات الملوثة ، لكن التثبيط الناتج عن *T. harzianum* كان أكبر باستخدام الراشح الفطري. في حين كان *P. ostreatus* له التأثير التثبيطي الأكبر على نمو الملوثات الفطريه وبكافة الطرق المستخدمه. بالرغم من الاختلافات في التأثير المثبط بين *T. harzianum* و *P. ostreatus* ضد فطر *A. niger* و *A. ochraceus* ، الا ان الفطرين الاحيائيين قد تميزا بنشاط مثبط وناجحً وقدرة على التنافس ضد هذين الفطرين .

جمهوريه العراق
وزاره التعليم العالي والبحث العلمي
جامعه بابل\كليه العلوم
قسم علوم الحياة

تشخيص الفطريات المنتجه للOchratoxin A في بعض الثمار المجففه في أسواق محافظة بابل وتوصيف خصائصه

رســـالة مقدمـــة إلـــى

مجلـس كليـة العلـوم ــ جامعـة بابل

وهـي جـزء مـن متطلبـات نيـل درجـة الماجستيـر فـي العلـوم

علوم الحيــاة

من قبل الطالبة

عبير هادي ناجي محسن عباس

(بكالوريوس علوم/ علوم الحياة – 2012)

بإشراف

ا.د. ابتهال معز عبد المهدي الحسيني ا.د. جواد كاظم عبود الجنابي

1442ه 2021