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Detection Effect Toxins Produced by Some Types of Fungi Isolated from Medicinal Plants

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***Abstract*---**Medicinal plants accompany many fungi belonging to different groups, including different types of zygomycotina, ascomycotina, deuteromycotina and yeasts (yeasts). Some of these fungi accompany the plant during its growth in the field and others contaminate weeds during Harvest and storage process. The fungi that infect crops were divided by Christensen (1965) into three groups. The first group includes the field fungi, which infect agricultural crops before harvest, and includes species belonging to the genera Alternaria, Fusarium, Helminthosporium, and Cladosporium. The second group is known as storage fungi, and includes fungi that attack crops agricultural crops during storage. It includes species of the genus Aspergillus and Penicillium, These fungi are characterized by the fact that most of them produce toxins (Mycotoxins), which cause diseases to humans and animals when consuming crops contaminated with them. The third group includes rot fungi (RootFungi) that grow on the remains of plant materials and include: Chaetomiumspp, Papulosporaspp, Fusarium graminearum and Sordaria spp and found that most of the toxin-producing fungi belong to storage fungi and a few of them belong to rotting fungi that have the ability to produce toxins. The study also showed the effects of aflatoxin produced by the growing fungi on some types of medicinal plants in experimental animals, a negative effect on the growth rate and the weights of their internal organs.

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***Keywords*---**Medicinal plants, aflatoxin, toxins, Aspergillus, Penicillium.

Introduction

Fungi are heterotrophic organisms that are widely distributed in nature in most environments, have diverse nutritional requirements, and feed in different ways. They may be saprophytic, parasitic, or symbiotic. found many fungi on stored leaves such as Rhizopusstolonifer, Aspergilliusflavus, Alternaria alternate, Fusariumsp. Penicilliumsp. Some fungi produce during the secondary also toxic byproducts that pose a threat to human and animal health. The most dangerous of these toxins are aflatoxins, which are poisons with a cumulative effect. Low doses of them are carcinogenic and mutagenic, while large doses are toxic and fatal. that infect the leaves during picking Also, during transportation or storage, it is caused by the fungus Aspergillus spp, especially the type A. flavus, and sometimes the fungus Penicillium spp. (Liu et al., 2018) It can grow and attack the fruits while they are in the field and after picking during the storage phase at low temperature 8–24 °C and a moisture content higher than 16% is considered Rhizopusstolonifer is a weak parasitic fungus on fruits during transportation and storage, which causes chrysalis leaf disease (Che *et al.*,2014) It also causes soft rot disease of sweet potatoes when stored. It is also used in the production of fumaric acid. It is also used to complete some steps in the production of cortisone. Also called breadmold, the mushroom body is composed of threads. A non-dividing branched fungus, i.e. a coenocyptic, that reproduces sexually by fusion of similar gametophytes. which requires a heterothallic to be a gametangial conjugation conjugation in which there are two physiologically distinct and harmonious fungal mycelium, one of which is symbolized by (+) and the other (-). Small with ovules or devoid of them and contain few spores. These capillaries are known as sporangiola It may contain a single preservative called Monosporesporangium, or the spore wall may fuse with the preservative wall, resulting in a single preservative. Image source Aspergilliusflavus of the fungus and Aspergilliusflavus of the fungus and of the fungi and of the fungi and of the fungi and data and data of various factors of man and cause what is known to be the causes of aspergillus infection or its occurrence. Aspergillosis, which is caused by some species of the genus Aspergilliussp, the body of the fungus is composed of fungal hyphae divided by septa and branched, and when they grow on the media, they give colonies of different shapes and colors. It reproduces asexually by forming conidiophores from the ends of the fungal hyphae, usually standing and not branching. It bears from its ends enlarged vesicular structures called vesicles, and at the base of the carrier, foot cells are formed. Also, it is noted that there are two rows of appendages or auricles arising from the wall of the vesicle and called sterijmala, which in turn carry chains of conidia of varying shape and size. During the life cycle, some types of them are known to reproduce sexually (rare). The perfect perfectstateTeleomorph that results in the formation of closed, spherical fruit bodies, and then it is called the genus Emericella, and the intermediate asexual phase is Anamorph, which represents its conidial state, and is called the imperfect phase, and the genus Aspergillus is under the section of Ascomycotina and the class of cystic fungi, Eurotiales, and the order Plectomycetes It causes rotting and damage to those fruits and fruits, and the

antibiotic Penicillin is extracted from it, and some of its types are used in cheese making and various chemical industries. This characteristic is difficult to distinguish between them with the naked eye, but there are phenotypic and structural differences upon microscopic examination. When asexual reproduction occurs, the conidiophores are branched, ending with phallic structures that carry chains of conidia and the absence of a vesicle, and thus it is possible to distinguish between the types of this genus from the previous sex (Liu et al., 2019).

Materials and working methods

Sample collection
Aspergillus

Aspergillus is a widespread fungus that includes many species that cause disease in humans and animals, spoilage and production of toxins as beneficial secondary metabolites through food fermentation. Source: The number of species belonging to the genus Aspergillus is more than (250) species, and new species are added from time to time, recorded in different regions of the world. They are more prevalent in cold cold regions. The genus Aspergillus is found in soil, on decomposing organic matter, on products such as fruits and seeds, and on animal waste Its species has the ability to grow and compete for food with the rest of the fungi, including the fungi that cause plant diseases, which gave the opportunity to use the fungus in biological resistance. The mycelium is well-formed and consists of branched, divided and multi-nucleated hyphae. Some species of the genus Aspergillus form small, stone bodies (Seclerotia) consisting of compact, thick-walled cells in the form of pseudo-barnica. Where these bodies arise when conditions are not suitable for the growth of the fungus A. niger (Liu et al., 2019). The mycelium of the genus Aspergillus consists of fungal hyphae (hyphae) that form thick-walled foot cells (FootCells) extending upward in the form of tubes that form non-polynucleated conidiophores. segmented, swells at its apex to form globose or hemi-spheric heads known as vesicles Nuclei, organelles, and cytoplasm move to it, after which one or two rows of tails are formed. In this case, the fungus is described as uniseriate, as in A.flavus. If there are two rows of calyxes, the genus in this case is described as biseriate, as in A. niger fungi. As for the conidia, they are either mononuclear conidia as in A. nidulans, or binuclear conidia as in A. niger fungi, or multinucleated as in A. nidulans. in A.repens.

Diagnostic and physiological characteristics of type A. niger

Mushroom colonies grow on the agar agar and potato extract agar and dextrose agar (PDA), as these colonies appear smooth or slightly wooly with a black color arising from the production of huge numbers of black conidia and the diameter of the colony reaches 5-6 cm after two weeks of growth, Some isolates are spherical or semi-spherical stone bodies with a diameter of 0.8 - 1.2 mm, cream-colored at the beginning of their formation, then yellow or orange after a period of time Conidia heads are characterized by being spherical in shape, but after increasing the numbers of conidia that form obtuse chains perpendicular to the surface of the crop, the conidia head acquires a radial appearance. Conidia carriers are

thick, smooth, transparent walls that may turn brown near their ends. Their dimensions range between 300-1500 micrometers long and 15-20 micrometers thick, ending with a spherical vesicle with a diameter of 40-80 micrometers, whose entire surface is covered by one row of primary flask structures in the beginning. With the advancing age of the crop, reconsideration of the second stage of secondary flaring structures, which are smaller and thinner than the primary flaring structures, and on top of which black to brown spherical conidia are generated with spiny spikes with a diameter between 4-5 micrometers. The species belongs to the group of fungi Deuteromycotina, and is classified within the family Moniliaceae to the order Moniliales Mushrooms secrete different enzymes and antibiotics that compete with microorganisms in the growth area The most polluting fungi of dried fruits such as grapes, apricots and figs are fungi belonging to the genus Aspergillus, followed by the genus Penicillium and then the genus Rhizopus, and some species of the genus Aspergillus are opportunistic fungi such as A. Parasiticus or A.flavus. Or A.niger, as it causes diseases in people with weak immunity called aspergillosis Its symptoms are similar to tuberculosis The fungus A.niger also produces many toxins that have an impact on human and animal health, such as aflatoxin, ochratoxin, gliotoxin, strigmatocystinoamodin, citrinin and cyclopizonic acid. Product Substance with a high toxic effect on newborn rats Newborn dilatation with bleeding of the duct connecting the stomach and intestines with changes in the liver and kidneys and this leads to the death of rats injected under the peritoneum, while it does not cause severe poisoning if the dose is given orally. A study conducted found that the weight of the rat in surgery was 12.3 mg, and also caused the uterus to gain weight due to the albino rat. While it was 153.6 mg in passenger animals. Many times fungi are toxic compounds to humans and animals during secondary metabolism Mycotoxins were first introduced in 1962 after a disaster. Famous turkeys that occurred in Britain. Mycotoxins are known as toxic secondary metabolites with carrot weights. And all of them are toxic, affecting the vital activities of the organism in various ways and may eventually lead to its death if it is ingested at levels that exceed the internationally agreed limits to allow its presence in raw materials, whether those used in human food or those used in the manufacture of feed for agricultural animals (Lopez et al., 2019).

Mycotoxins

Many types of fungi produce compounds that are toxic to humans and animals through secondary metabolism called Mycotoxins are known as toxic secondary metabolic compounds with relatively low molecular weights. Several hundred types of mycotoxins have been discovered to this day, the most important of which are aflatoxins, trichothecens, ochratoxins, zearalenone, and all of them are toxic and may affect the vital activities of the organism in various ways. Ultimately, it will perish if it is eaten at levels that exceed the limits that have been internationally agreed to allow its presence in raw materials, whether those used in human food or those used in the manufacture of feed for agricultural animals. A number of accidents were recorded in Japan in the year 1891, the impact of eating rice contaminated with fungi. Toxicosis Yellow Rice as a disease caused by contamination with toxins produced by various types of fungi such as Aspergillus and Penicillium

Toxin-producing fungi

The types of fungi that reach six genera of fungi and are the most widespread and characterized by their ability to produce mycotoxins in high concentrations and live in diverse environments and with simple growth requirements of temperature and humidity of the genera is Aspergillus spp. and Penicillium, Fusarium, Claviceps, Stachybotrys, and Neotyphodium [] While there are other genera that can be renaming toxins, varying such as Alternaria, Rhizopus, MucorTrichothecium, Trichoderma, Rhizoctonia and others The three genera of fungi Aspergillus, Penicillium, and Fusarium are the most common mycotoxins in granaries. Aspergillus and Fusarium attack the grain in the field and persist during storage. The biological effects of mycotoxins in the living body vary according to the type of toxin and the sex and type of mushrooms that secrete the toxin, and that the most important mycotoxins that pose a danger from the beginning and return are mainly strontino, patulin, and ochratoxin produced by the types of fungi Penicillium spp. Trichothycinato-zeeralenone is a source of the fungus spp. Fusarium.

Addition of toxicity to mycotoxins

Biological and toxicological properties differ, as toxins are considered within the physiological processes carried out by the cell in the nervous system, circulatory system, internal system and internal organs such as the liver, kidneys, heart and spleen. And another entry (Li et al., 2018) and the Internation out of your check out out Mycotoxins and their effects on human health or non-allied agricultural animals, including their origin from studies and tests of animals, animals, animals and their animals and within them is a matter of a great deal of danger**,** The various types that have been diagnosed in addition to those diagnosed by infecting humans, animals and plants. Mycotoxins play an important role in causing various health effects. They are either present in food and pose a significant danger to the consumer when eating food contaminated with it, or they are present in the air and cause serious health problems when inhaled by the organism. This method is called direct methods.

Effect of mycotoxins on body tissues

The liver is the target organ in case of poisoning with most mycotoxins, and it may be in other organs. Diseases resulting from ingestion of feed contaminated with mycotoxins, including aflatoxins, range from acute hepatitis. (Hormorage) and bur changes (lipid changes) are evident in the liver, (hepatic necrosis of lobules) (acoustic necrosis of the lobules) while exposure to various forms of these toxins. Hepatocytes (hepatocellular carcinoma) In a study of the effect of aflatoxins B1, B2, G1, G2 on both the liver and spleen tissue in rabbits, it caused cellular infiltration and necrosis in the liver cells with asymmetry in the liver lobes. These toxins also affected the spleen tissue, causing its congestion and the occurrence of red pulp aplasia. . Also, aflatoxins may cause severe changes in the chromatin material and cause an abnormal increase in the size of the nucleus accompanied by vascular congestion of the parenchymal tissue of the liver as well as effects on the kidney tissue in white mice.

The main types of mycotoxins

Diagnostics classify mycotoxins on the basis of the affected organ. Toxins that affect the liver are called hepatotoxins, neurotoxins, and nephrotoxins. While specialists in life sciences put these toxins in groups according to their general effect, for example, they are called carcinogens, mutagenes, and allergenes. As for microbiologists, they classify mycotoxins on the basis of the fungi that produce them. For example, those produced by Aspergillus are called Aspergillus toxins, and those produced by Penicillium are called Penicillium toxins. While there is a classification that depends on the chemical nature of the poison, and this classification works by the chemists, who divide the mycotoxins into groups, for example, the group of mycotoxins that contain Coumarin lactones, the group of polyketides, and the group of mycotoxins derived from amino acids. The following is a brief summary of some mycotoxins and their biological effects:

Aflatoxins:

They are carcinogenic and toxic metabolic compounds and are considered one of the most dangerous types of mycotoxins for humans and animals. They also belong to the Difuranocoumarin group, the most important of which are Aspergillus species such as A. flavus, A. parasiticus and A.nomius, and species of the genus Penicillium such as P.citrinum and P.frequentans. The researcher Meerdink is the first to isolate and extract four types of aflatoxins produced by A.flavus and A.parasiticus in the form of fluorescent spots called B1, B2, G1, and G2, where the letters indicate the color of the fluorescence that the spots appear on the chromatographic plates when examined under ultraviolet rays, The letter B symbolizes the blue color, the letter G symbolizes the green color, and the numbers 1 and 2 symbolize the transfer factor (Rf) rate of flow shown by spots on TLC sheets (Wild et al., 2010). Two other types of aflatoxins have also been identified, they are M1 and M2 which are called milk toxins. Aflatoxins B1 and B2 are produced respectively by hydroxylation in dairy animals. Milk toxins 1M and 2M are excreted at a rate of approximately 1.5% of the aflatoxin B consumed.

After that, the aflatoxins were extensively studied and divided according to the degree of their toxicity and the percentages of their presence in food and feed Aflatoxin B1 is the most dangerous and is present in higher concentrations than the rest of the toxins in the natural contamination of products, followed by aflatoxin G1, B2 and G2. The structural formulas of aflatoxins B1 and G1 were previously determined, while the structural formulas of aflatoxins B2 and G2 were previously determined. It was found that A. flavus produces aflatoxins B1 and B2 and A. parasiticus produces B1, B2, G1 and G2 (Chen et al., 2014).

Aflatoxins are characterized by the intensity of the fluorescence emitted by them when exposed to ultraviolet rays, and this characteristic makes it possible to detect their presence at very low levels for each of the detection spots installed on the chromatographic examination plates. Stable at temperatures below 200° and in the absence of light, Aflatoxins have received a lot of attention because of their effective effect on humans and animals. They are one of the highly toxic food pollutants that can enter the food chain from the field to reach the consumer, as well as being one of the most important causes of liver cancer in humans. It also

has suppressive effects on the immune system in addition to its mutagenic effects and causing birth defects.

Genetically, these toxins have effects at the molecular level, as it was explained Cocker et al that aflatoxins are responsible for teratogenes and mutagenes, by inhibiting the building of DNA and RNA, as well as toxic effects on genes. Amino transcription and thus inhibition of protein synthesis

Also, aflatoxins cause severe effects in the chromatin material and cause an abnormal increase in the size of the nucleus accompanied by vascular congestion in the parenchymal tissue of the liver, as well as clear effects on the kidney tissue in white mice. And the amount of hemoglobin. She also indicated in her study on the effect of aflatoxin toxins on some physiological and biochemical parameters in female albino rats that they caused a decrease in the levels of FSH and LH. Here is the chemical formula of aflatoxins B1, Ochratoxins:
Ochratoxins are secondary fungal metabolic compounds of similar chemical structures, which are classified according to their biosynthetic origin as a group of pentaketides within the polyketides produced by fungi A. ochraceus, A.ostinus, and A. ochraceus. meleus and some Penicillum mushrooms such as P. verruosum, this toxin is found in cereals, coffee and dried fruits, and can also be found in the meat of animals fed contaminated feed (Frisvad et al., 2018)

Materials and working methods
Sample collection

30 samples of medicinal herbs belonging to three species, namely leaves (Menthalogifolia (L)) and fenugreek seeds (Trigonllafoenum - graecun (L)) were collected from Al-Attarin market in Nineveh governorate.
Used agricultural media and methods of preparation
The following media was used for the purpose of isolating fungi from medicinal herbs samples

Growth on medium SDA
The samples were planted on the medium of the saprod dextrose akar according to the method

Isolation and identification of fungi
The direct plate method was used to isolate the fungi accompanying the medicinal herbs samples with a weight of 10 g from each plant sample separately, then superficially sterilized with 10% sodium hypochlorite for 5 minutes and then washed with distilled water Transfer 100 pieces of each cinnamon and thyme leaves and 100 fenugreek seeds to sterile filter paper for drying.

Then the cuttings of each plant were distributed on the surface of the used plant media by 5 pieces / plate, and after the completion of planting the plant parts, the plates were incubated at a temperature of 25° C for 7 days Abramson (Abramson et al., 2017).The dishes were examined 7 days after transplantation for the purpose of diagnosis of colonies growing on the culture media and continued to monitor the dishes for a period of 4 weeks This is to allow the growth of cystic fungi and their appearance, which is delayed in growth after incubation, and the

initial examination of the dishes was carried out, and then the fungi were isolated using the needle on plates containing the agricultural medium (Direct plate).

For the purpose of studying the characteristics of the isolated fungi and accurately diagnosing them, they were examined under a light microscope (CompoundMicroscope) By preparing glass slides fixed with lactophenol containing the methyl blue dye. Undoubtedly, in our understanding of the effects that mycotoxins can have on overall health (Han et al., 2017).

Diagnosis of the fungus A.flavus isolate used in the study

The diagnosis of A.flavus isolate growing on solid (PDA) medium was confirmed
1) The incubated at a temperature of 27˚C for seven days by growing it on the following and special agricultural media
Diagnosing Aspergillus species:
1- Czapek Yeast Extract Agar
2- Medium Malt Extract Agar (MEA)
3- Medium Glycerol Nitrate (G25N) 25% Glycerol Nitrate
Regular shaped discs were obtained from the fungal colony with a diameter of (0.5) cm using a Cork Borer after sterilization with 70% alcohol Then the combustion and cooling, and using a deep and cooled pollination needle, the disc was lifted and placed in the center of a glass dish containing one of these three agricultural media At a rate of three replicates for each culture medium under sterile conditions, then the dishes were incubated at temperatures 5, 25 and 37° C for a period of seven days after which the diagnosis was made Fungi depending on the growth shape, colony color and diameter, and the presence or absence of secretions and pigments, and based on the approved taxonomic keys

Diagnosis of aflatoxin isolate A.flavus secretion
The secretion of aflatoxin toxin by the fungus A.flavus was diagnosed by growing it on the following two media
1- middle Aspergilius flavus and Parasilisus Agar(AFPA)
It is a differential medium for the detection of the aflatoxin-producing strain that gives a yellowish-orange and bright color in the background of the culture medium after 48 hours of incubation at 30˚C temperature
2- middle Aflatoxin-Producing Adility Mediu˚(APA)
It is a specialized medium to detect the strains of the two parties A. Parasiticus A.flavus The producer of aflatoxin toxins by taking advantage of the ability of ultraviolet rays to induce aflatoxins to fluoresce at the wavelength of 365 nm If the fungal colonies growing on this medium for 7 days and at a temperature of 27°C give blue fluorescence under an ultraviolet lamp in a dark room(Chen.,*et al* 2014).

Vital tests
Experimental animal source
Experimental animals were obtained from the College of Veterinary Medicine at the University of Mosul and are Mus musculus egg mice

Breeding and breeding experimental animals

Experimental mice were bred and multiplied in the experimental animal breeding room in the Department of Pathological Analysis, Mosul Technical Institute, Northern Technical University Under a temperature of 17--21°C, a light duration of 12 hours of light and 12 hours of darkness And that is in special plastic laboratory cages for breeding mice with metal mesh covers, dimensions 30 cm in width and 13 cm in height Northkent Plasstic Cages ltd.England The floors of the cages were spread with fine sawdust and free of materials against termites, taking into account changing it and washing the floors daily. They were also prepared with locally prepared food according to the following ingredients

- Wheat flour 30%.
- Barley flour 24.5%
- Corn flour 22.5%
- Soybean flour 15.2%
- Concentrated animal protein 7.22%
- table salt 0.4%
- lime 0.18%

The food for the mice was prepared according to the above ingredients by taking a quantity of the forage and adding a little water to it and kneaded by hand to become cohesive, then it was cut in the form of pellet cylinders with a length of approximately 2 cm by a special machine Sexually mature experimental mice were placed 4:1 (male: female), taking into account that males switched between cages in the event that pregnancy did not occur after 10 days or more, and males were isolated from females after Ascertaining the occurrence of pregnancies, then distributing pregnant females in separate cages with one female per cage to provide suitable conditions for childbirth and breastfeeding, and equipping them with birthing beds made up of soft tissue paper pieces (Kleenex), and the youngsters were weaned at the age of 21 days by isolating them from the mothers and thus ready for the vital tests (Nafade et al., 2018).

Design of bioassay experiments

Biometrics trials were designed in a Complete Randomized Design. Gender, age, and weight were standardized across all parameters. Experiments as much as possible. If all experimental mice were males only, and their age was 21-25 days Mice of each experiment were chosen randomly, taking into account the closeness of their weight. Each treatment was designed with three replicates (three mice) and the animals of each treatment were placed in a culture cage microscopically with fine sawdust and provided with artificial feeding bottles Local food and food, with the necessary information installed on each cage, which is the date of birth, the date of the start of the experiment, the date of its end, and the type of treatment (Chen et al., 2014).

Effect of aflatoxin on experimental animals
Preparation of aflatoxin

The aflatoxin was prepared according to the method of Shotwell et al. (1966,) by soaking rice in distilled water for an hour, then extracting from the soaking water and placing it in a closed-mouthed glass flask. minutes and then inoculated with

spores of A. flavus under sterile conditions and incubated at temperature 30˚ C for seven days in a Germany, Memert incubator, taking into account the daily movement of the beaker and the addition of sterile distilled water with a diffuser of 80 Tween during the incubation period, and after the growth of Mushrooms on rice grains were sterilized again with a sterilizer device at a temperature of 121˚C, and a pressure of 15lbs/in2 for 10 minutes on a thinly applied ceramic tray and dried in the oven

At a temperature of 60°C, it was ground after drying to obtain a powder containing crude aflatoxin Aflatoxin was investigated in medicinal plant powder Weigh 100 g of each type of medicinal plant powder contaminated with aflatoxin, add 300 ml of methanol to it and mix well for 5 minutes, then filter the mixture through dissection paper Then the precipitate was discarded and 150 ml of the filtrate was taken into the separation funnel and 50 ml of gasoline was added to it. Two layers were formed by the tap in the separating funnel. The lower layer was neglected As for the upper layer, it was concentrated by volatilization, and before it dried completely, several drops of the concentrated extract were taken on a filter paper and examined under ultraviolet rays

Ultraviolet rays were used by using an ultraviolet lamp (Hanovia-England) with a wavelength of 365 nm and in a dark room. The blue brilliance of the drops of the concentrated extract was observed Evidence that the concentrated extract formed after drying the sample is aflatoxin B1. The aflatoxin was quantified for each type of contaminated medicinal plant powder in the Central Laboratory \ Erbil by ELIZA method.

Preparing food contaminated with aflatoxin

The diet containing aflatoxin B1 was prepared at two doses of 5 and 7.5 mg/kg of the weight of the basic food for mice (Al-Jubouri, 1994) by adding contaminated rice powder.

With aflatoxin to the main food, mice were fed unlimited amounts of food for all treatments, each treatment contained three replicates, and each replicate was one animal. The aflatoxin feeding continued for four weeks, and the tested animals were weighed weekly.

Renewing the diet according to the average weight of the animals for each week

Animal anatomy experiments

Four weeks later, the treated animals were autopsied after killing them with chloroform. After the autopsy, changes in the appearance of a group of internal organs, represented by the liver, kidney and spleen, were observed in terms of change in size, shape and color. Comparing the internal organs of animals with the comparison treatment, and then removing each organ separately and weighing it, then calculating its weight per 100 g of body weight

Statistical analysis

The results of the current study were analyzed according to the Duncan polynomial test at a probability level of 5%, and this was implemented using an electronic calculator (Zhang et al., 2017).

Results and discussion

The results of the study, which was conducted on medicinal herbs in Mosul Governorate, showed a distribution of fungi on herbs throughout the year and with different prevalence rates, which indicates the presence of pathogenic fungi in the stores, according to the places and methods of storage in the stores. (Frisvad.,et al 2018). The percentages of the frequency of fungi differed from one fungus to another, and this may be due to the difference in herbs, as well as the type of plant and the factors available for the occurrence of infection As for the distribution of the frequency of fungi over the months of the year, the lowest percentage was recorded in the month of January for the decrease of the second degree to a level close to zero percentile, which is less than the minimum degree in which vital activities stop Most of the fungi, which does not encourage any infection with fungi, due to the lack of appropriate environmental conditions and the absence of fungi that can withstand very low temperatures and can cause injury, as well as the lack of appropriate paper sometimes(Han et al., 2017).

Then there were infections of the weeds stored in the month of February, which came from the impossible initial vaccination due to unfavorable conditions (Hou et al., 2018). Then the number of leaf infections increased with fungi in the month of March, due to a rise in temperature of approximately 25 The reason for the distribution of fungi in the stored herbs over the months of the year in these different proportions is due to the environmental conditions and the difference in the fungi in their tolerance to those conditions. (Zhang et al., 2018)

Table 1
The percentages of the frequency of fungi in the alabaster herb

	isolated fungi	Frequency ratio
1	*Aspergillus flavus*	8.2
2	*A.niger*	8
3	*Alternaria alternate*	-
4	*Fusarium oxysporum*	3
5	*Penicillium*	1.25
6	*A.terrus*	2

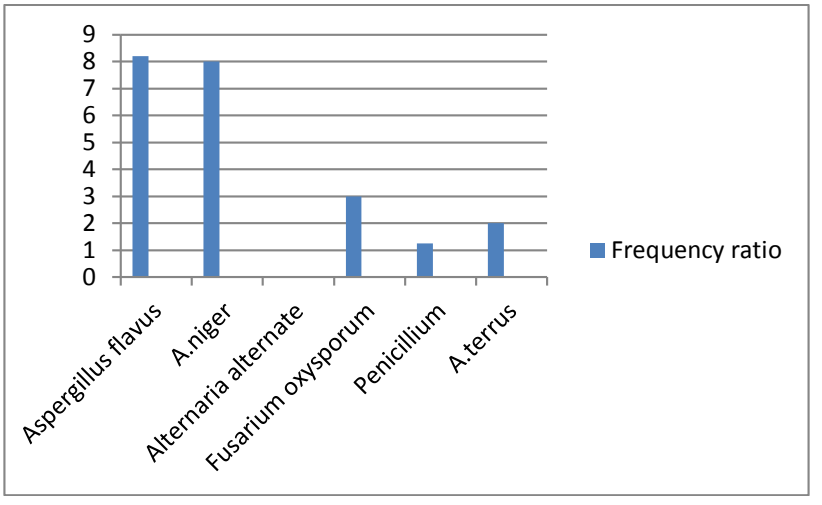


Table 2
Frequency ratios of fungi in nettle herb

	isolated fungi	Frequency ratio
1	*Aspergillus flavus*	5
2	*A.niger*	4.2
3	*Alternaria alternate*	-
4	*Fusarium oxysporum*	2
5	*Penicillium sp*	-
6	*A.terrus sp*	-

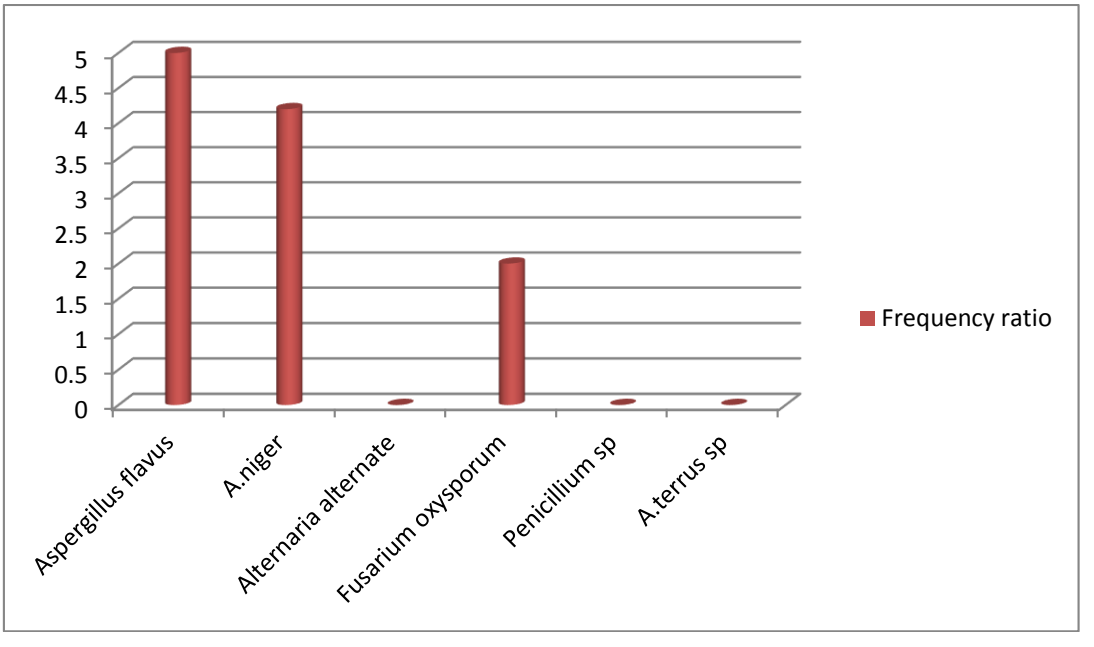


Table 3
Frequency ratios of fungi in the mint herb

	isolated fungi	Frequency ratio
1	*Aspergillus flavus*	4
2	*a.niger*	2
3	*Alternaria alternate*	1
4	*Fusarium oxysporum*	-
5	*Penicillium*	1.77
6	*a.tesrrus*	1

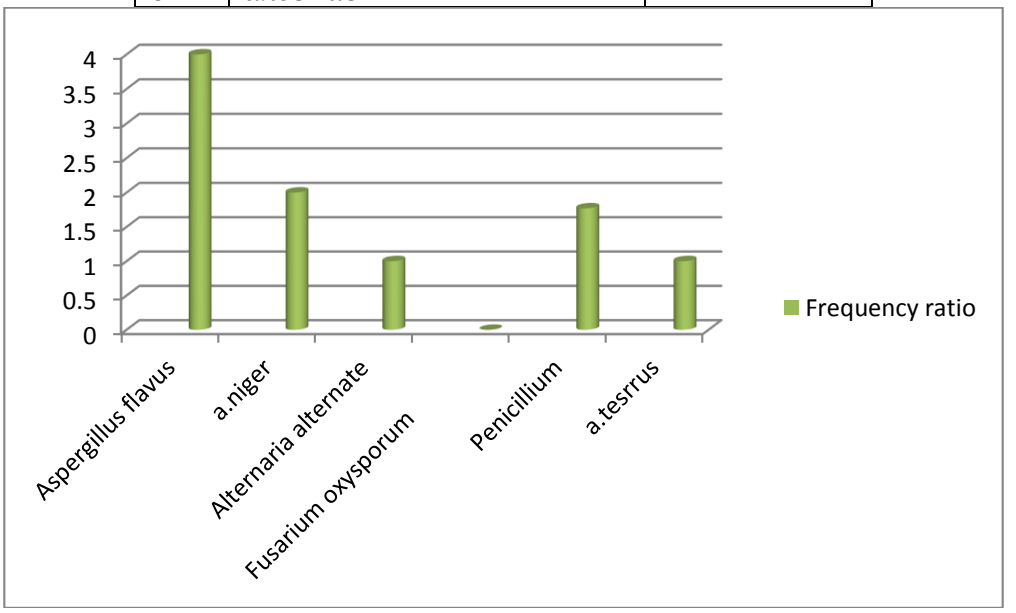
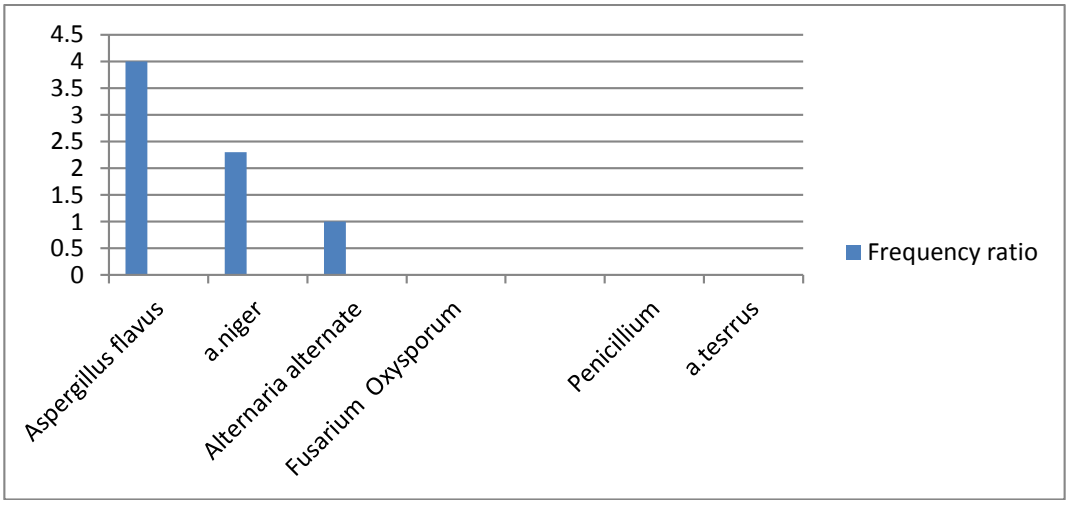


Table 4
Frequency ratios of fungi in anise herb

	isolated fungi	Frequency ratio
1	*Aspergillus flavus*	4
2	*a.niger*	2.3
3	*Alternaria alternate*	1
4	*Fusarium Oxysporum*	-
5	*Penicillium*	-
6	*a.tesrrus*	-



Mycotoxins are secondary fungal metabolites, toxic to humans, animals and plants. Among the hundreds of known mycotoxins, aflatoxin, citrinin, patulin, penicillin Acid, Tenoasonic Acid, Okratoxin A, Cytocalcin, Dioxinevalinol, Fumonisin, Fuzarin C, Fusaric Acid and Zeeralenone are the most contaminated of cereal grains The majority of mycotoxins in these groups are produced by three fungal genera: Aspergillus, Penicillium, and Fusarium. These metabolites mainly affect the quality of the seeds Germination, vigor, vigor of seedlings, growth of roots and cloptil. In addition, since the fungi responsible for the production of these mycotoxins are often indoor plants that infect and colonization of living plant tissues, accumulation of mycotoxins may occur in plant tissues and experimental models. A combination of innate and adaptive immune recognition allows the host to eliminate invading pathogens from the body. However, imbalances in the immune balance often facilitate microbial infection (Ismail et al., 2018). On Despite the widespread effects of mycotoxins on health, our understanding of toxin-mediated modulation of immune responses is incomplete Also, the biological effect of mycotoxins on health is somewhat large, then the detection of aflatoxin and ochratoxin gives the types of mycotoxins from the disease that suffer from fungal infection, as well as toxins can be acquired by ingestion, which can cause somewhat acute intoxication, causing breakdown of mucosal barriers, stimulating cell death, and suppression of microbial immune cell function(Priyanka et al., 2017).

4-2 Diagnosis of aflatoxin isolate of the fungus a.flavus

It was confirmed that A. flavus isolates secreted aflatoxin through Showing a yellowish-orange and bright color in the background of the developing colony on the middle Aspergillus flavus and Parasiticus Agar, and this agrees with what he found (Kumar et al., 2016)

The aflatoxin-secreting isolate of the fungus

A flavus shows a yellowish-orange color in the background of the fungal colony when grown on a medium (Tamura, 2014) AFPA also showed isolation when

grown on (APA) medium Aflatoxin-Producing Adility Medium - Fluorescent bluish flash under x-ray lamp Ultraviolet light with a mixed length of 365 nm when exposed to it in a darkened room that loneliness (WHO., 2018)
The aflatoxin-secreting fungus A flavus exhibits a fluorescent glow under ultraviolet light with a wavelength of 365 nanometers.
Effect of aflatoxin on growth rate and internal viscera weight of male white mice for a whole month that loneliness (Tripathi et al., 2014).

Table 4
Effect of aflatoxin B1 on growth rate and internal visceral weights of male white mice

Transaction	Weight rate Primary(GM)	weight place Final (GM)	Rate of increase in weight within four weeks (gm)	The weight of the internal viscera is g / 100 g of weightthe body		
				liver	the kidneys	spleen
Aflatoxin10 mg/kg food	10.5	18.2	8.2B	6.92A	2.40A	0.70A
Aflatoxin 5 mg/kg food	8.2	19.25	11.05B	6.35AB	1.56A	0.40A
Comparison	7.07	21.29	12.71A	4.71B	1.22A	0.23A

Table (1) shows the effect of aflatoxin B1 at the two doses 5 and 10 mg/kg of food weight On the growth rate and internal viscera weights of male mice aged 21-.25 per day for four weeks Where the treatment with aflatoxin in both doses led to a significant decrease in the average weight of mice The treatment than the comparison treatment if the rate of weight gain was 9.63, 10.06 and 12.71 g for each of The dose 5, 10 and the comparison treatment, respectively (Tripathi et al., 2017) also had a significant increase in the average liver weights In animals treated with aflatoxin 10 mg compared to the comparison and this is consistent with what was found by all Where it was found that the addition of concentrations 2.5, 5 and 7.5 mg/kg diet of aflatoxin to the staple diet of rats and mice for 28 days may cause A decrease in growth, as well as in the rate of weight gain, in addition to an increase in both liver weights And the kidneys and spleen,(Deshpande et al ., 2016) as confirmed by the fact that the addition of aflatoxin (1 parts per million) to the diet of chicken chicks caused a decrease in growth and a decrease in the efficiency of food conversion as well Enlargement and significant increase in the weight of the liver that add Feeds contaminated with aflatoxin at a dose of 3.5 and 7.5 ppm consumed by male chicks Meat broilers at the age of one day for three weeks led to a significant effect in reducing the average weight The body and the enlargement of the internal viscera and increase their weight, as the aflatoxins have a significant effect in reducing Weight gain(Taye et al., 2018) feed intake and metabolic efficiency (Picture 12) shows the effect of aflatoxin dose 7.5 mg/kg of food weight on the body The liver, where we notice its enlarged size, muddy abdominal texture and pale yellowish color due to Fat leaching of cells, as aflatoxin causes a significant decrease in cholesterol levels And triglycerides in the blood serum, and it affects the fat content of the liver, as it decreases In the

digestion of fats, fat cells accumulate in the internal organs, especially the liver (Tanaka et al., 2019). The researchers attributed this to the possibility of insufficient efficiency of the suprarenal gland by the effect of aflatoxin, which is responsible for Decreased release of free fatty matter from adipose tissue into the blood, as well as Aflatoxin causes necrosis, damage, and then death of liver cells, and this is due to the metabolic compounds of these Toxins that interact negatively with cell proteins, causing a marked decrease in liver function Aflatoxin impairs the biosynthesis of proteins by forming complexes with DAN (Wielogorska et al., 2019), RAN proteins also inhibit the activity of RAN polymerase, thus leading to Significant decrease in DAN level and liver total protein content, in addition to its reduction Serum ALT aminotransferase Numerous studies have shown that aflatoxin B1 has a mutagenic and carcinogenic effect.

Carcinogenic occurs through the metabolic processes that take place inside the body, including Epoxidation process, which results in the compound 8,9-epoxide, which is one of the compounds Carcinogenic and the most toxic among the metabolites of aflatoxin B1, the genetic mutation occurs when it is linked to of the 8,9-epoxide complex with DNA at the N7 position of The nucleotide base guanine is at the third position of codon 249 in Tumor suppressor gene P53, causing the gene to break down and the mutation to appear genetics and liver cancer. (Deshpande et al., 2014)

References

Abramson D., Mills J.T., Marquardt R.R., Frohlich A.A.(2017) Mycotoxins in fungal contaminated samples of animal feed from western Canada, 1982–1994. Can. J. Vet. Res. ;61:49–52.

Chen X.P.(2014) Analysis on the problems of 3477 batches Chinese herbal pieces by our hospital acceptance. *China Med. Her.* ;11:139–142. doi: 10.1007/s00052-014-0035-6.

Deshpande KS and Gajewar DS: (2016) Effect of toxic metabolite of Helminthosporium longirostrata on germination of M35-1 Sorghum. Indian Journal of Agricultural Science, 46 (12), 1976: 584-588. [8] Kandhare, A. S.. Mycotoxic effects of seed-born fungi on seed health of black gram. Journal of Plant & Agriculture Research, 1(1), 1-3.

Frisvad J.C., Hubka V., Ezekiel C.N., Hong S.B., Nováková A., Chen A.J., Arzanlou M., Larsen T.O., Sklenář F., Mahakarnchanakul W., et al.(2019) Taxonomy of section and their production of aflatoxins, ochratoxins and other mycotoxins. Stud. Mycol. 93:1–63. doi: 10.1016/j.simyco.2018.06.001.

Han Z., Ren Y.P., Zhu J.F., Cai Z.X., Chen Y., Luan L.J., Wu Y.J.(2017) Multianalysis of 35 mycotoxins in traditional Chinese medicines by ultra-high-performance liquid chromatography-tandem mass spectrometry coupled with accelerated solvent extraction. J. Agric. Food Chem. ;60:8233–8247. doi: 10.1021/jf301928r.

Hassan-Kadle, M. A. (2017). The Diagnostic Challenges, Possible Etiologies and Lack of Researches of Hepatocellular Carcinoma in Somalia. Open Journal of Gastroenterology, 7(03), 115.

Hou L.L., Zhou X., Gan F., Liu Z.X., Zhou Y.J., Qian G., Huang K. (2018) Combination of selenomethionine and N-acetylcysteine alleviates the joint toxicities of aflatoxin B1 and ochratoxin A by ERK MAPK signal pathway in

porcine alveolar macrophages. J. Agric. Food Chem. ;66:5913–5923. doi: 10.1021/acs.jafc.8b01858.

Ismail A, Goncalves BL, de Neeff DV, Ponzilacqua B, Coppa C, Hintzsche H, Sajid M, Cruz AG, Corassin CH, Oliveira CAF. . (2018) Aflatoxin in foodstuffs: occurrence and recent advances in decontamination. Food Res Int;113:74–85

Kumar S., Stecher G., Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 2016;33:1870–1874. doi: 10.1093/molbev/msw054. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Li L, Ma X, Pandey S, Fan A, Deng X, Cui D.(2018). Bibliometric analysis of journals in the field of endoscopic Endonasal surgery for pituitary adenomas. J Craniofac Surg.;29(1):e83–7.

Liu C.M., Qin J.A., Dou X.W., Yang M.H., Sun X.B. (2018) Extrinsic harmful residues in Chinese herbal medicines: Types, detection, and safety evaluation. *Chin. Herb. Med.* ;10:117–136. doi: 10.1016/j.chmed.2018.02.002.

Liu Y.P., Ma H.C., Zeng Q.Z. (2019) Quality evaluation of 733 batches of Chinese herbal medicines. *World Latest Med. Inf.* ;19:238–239.

Lopez-Munoz F, Tracy DK, Povedano-Montero FJ, Breedvelt J, Garcia-Pacios J, Fernandez-Martin MP, Rubio G, Alamo C. (2019).Trends in the scientific literature on atypical antipsychotic drugs in the United Kingdom: a bibliometric study. Ther Adv Psychopharmacol.;9:2045125318820207.

Nafade V, Nash M, Huddart S, Pande T, Gebreselassie N, Lienhardt C, Pai M. A (2018).bibliometric analysis of tuberculosis research,.;13(6):e0199706.

Priyanka S.R., Venkataramana M., Kumar G.P., Rao V.K., Murali H.C.S., Batra H.V. (2017) Occurrence and molecular detection of toxigenic Aspergillus species in food grain samples from India. J. Sci. Food Agric. ;94:537–543. doi: 10.1002/jsfa.6289.

Shi Q.N. *Master's Thesis.* Guizhou University; Guizhou, China: Jun,(2017). Evaluation of Moldy Fungus and Mycotoxin Contamination in Coix Seed.

Srinivas, A., Pushpavathi, B., Lakshmi, B. K. M., & Shashibhushan, V. (2017). Detection of seed borne mycoflora of sunflower (Helianthus annuus L.). Pharma Innov. J, 6, 256-259.

Tamura K., Nei M., Kumar S. (2014) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. USA. ;101:11030–11035. doi: 10.1073/pnas.0404206101.

Tanaka B, Maeda JA, Plazas I.(2019) Fungal microflora con seeds storage environment. Scientia Agricola.; 58:501-508.

Taye, W., Ayalew, A., Dejene, M., & Chala, A. (2018). Fungal invasion and mycotoxin contamination of stored sorghum grain as influenced by threshing methods. International Journal of Pest Management, 64(1), 66-76.

Tripathi RK:(2012) Head fungi of Sorghum, phototoxic and other effects on seed germination. Indian Phytopathology,: 499-501.

WHO (2018), Food Safety Digest – Aflatoxins, Department of Food Safety and Zoonoses, https://www.who.int/foodsafety/FSDigest_Aflatoxins_EN.pdf,

Wielogorska, E., Mooney, M., Eskola, M., Ezekiel, C. N., Stranska, M., Krska, R., & Elliott, C. (2019). Occurrence and human-health impacts of mycotoxins in Somalia. Journal of agricultural and food chemistry, 67(7), 2052-2060.

Wild CP, Gong YY. (2015) Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis.;31(1):71–82

Zhang J, Chen X, Gao X, Yang H, Zhen Z, Li Q, Lin Y, Zhao X. (2017). Worldwide research productivity in the field of psychiatry. Int J Ment Heal Syst.;11(1):20.
Zhang L., Dou X.W., Zhang C., Logrieco A.F., Yang M.H.(2018) A review of current methods for analysis of mycotoxins in herbal medicines. Toxins. ;10:65. doi: 10.3390/toxins10020065.