

Chemical Profiling and Toxicology Assessment of *Talaromyces columbinus* in Stored Grains of Karbala

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Abstract: The objective of the investigation is to scrutinize the domain of *Talaromyces* fungi distribution in warehoused grains solely, with the aim of verifying the existence of mycotoxins typified by aflatoxins using chemical Approaches. This is due to the grave hazard they pose once they infiltrate the market and are consumed by the end user, whether it be an individual or an animal. The results of the field assessment of grains, conducted in Karbala Governorate, Iraq, revealed the existence of *Talaromyces* fungi in varying percentages across the different types of grains examined. This revelation affirms the prevalence of *Talaromyces* in the local markets, thereby confirming its reach to the end consumer. Furthermore, owing to the majority of the identified species being aflatoxin-laden, this fungus presents a serious peril to consumers, inducing afflictions including but not limited to cancer, liver damage, and kidney damage, amongst others.

Introduction

In the past few years, there has been a significant increase in the number of species within the *Talaromyces* sect. *Talaromyces*, resulting in a fresh understanding of the variety present within the genus. (1) Due to this expansion, a newfound comprehension of the diversity within the genus *Talaromyces* has emerged, except for *Talaromyces pinophilus* and its closely related species. (2) Notably, the examination of the recently found species did not include *Talaromyces pinophilus* and its closely related species. Instead, the main emphasis was placed on the other members of the genus. (3) In addition to the intrinsic curiosity surrounding these fungi, certain *Talaromyces* species possess essential qualities for safeguarding plants. (4) Moreover, ongoing research is being conducted on the human infection caused by *Talaromyces marneffeii*, further highlighting the significance of these fungi beyond their ecological role. (5) *Talaromyces* is a diverse genus found in the Order Eurotiales. It includes numerous species with a wide global distribution and a variety of ecological niches. Their prevalence is ubiquitous, permeating the air, soil, plants, and interior habitats [6, 7]. According to sources [8, 9], some individuals have the ability to flourish in challenging environments such as extreme temperatures (both high and low), high levels of salt or sugar, low acidity, or limited oxygen levels. The three genera's species are mainly environmental saprobes [8, 9], and their primary ecological role is the breakdown of organic materials. [6]. *Talaromyces* species hold significant commercial, biotechnological, and medical value and thus bear significant societal implications. The food industry, in particular, relies on various species for the production of fermented foods such as cheeses (e.g., *P. roqueforti*) and sausages (e.g., *P. nalgiovense*). These fungi possess exceptional degradative properties, making them indispensable in the field of biotechnology, where they have been utilized in enzyme production [10, 11]. Additionally, *Talaromyces* are prolific producers of numerous secondary metabolites (extrolites),

some of which exhibit potential as antibiotics and medicines or as primary compounds in prospective therapeutic candidates with pharmacological or biological properties [12]. Nevertheless, other species, such as *P. citreonigrum* and *T. macrosporus*, exhibit decomposer behaviour, leading to crop degradation both before and after harvest. Furthermore, several of these species responsible for agricultural spoilage also produce mycotoxins [13]. With the exception of the thermally dimorphic fungus *Talaromyces marneffeii* (formerly known as *Penicillium marneffeii*), other pathogenic strains of *Penicillium* and *Talaromyces* species are generally not known to cause diseases in humans or animals. The aforementioned fungus is widely distributed in Southeast Asia and possesses the ability to elicit systemic infections, especially among patients with weakened immune systems, such as those afflicted with HIV [14] or those with diminished cell-mediated immunity [15]. The fungus in question holds immense significance due to its remarkable ability to generate aflatoxin, a highly potent carcinogenic secondary metabolite, from which it derives its name. Aflatoxin has been known to contaminate a variety of crops, both pre- and post-harvest, with oilseeds such as corn, peanuts, cottonseed, and tree nuts, as well as cereals, soybeans, figs, and almonds, being particularly susceptible. According to the United Nations' Food and Agriculture Organization (FAO), the worldwide impact of such contamination on food and feed is staggering, resulting in direct losses of up to US\$225 million annually, with the indirect effects being impaired human and animal health. (16).

Material and Methods

Solation and Identification of Fungi Associated With Some Local Nuts and Grains

A segment of every individual sample, comprising nuts and grains, underwent surface sterilization using a 2% concentration of sodium hypochlorite for a duration of two minutes. Subsequently, the samples were rinsed using sterile distilled water and transplanted onto a blotting paper-laden container dish to facilitate drying. Thereafter, they were planted in a nine-centimeter-diameter container on a sterilized medium (PDA) at a specified temperature. Upon cooling, the samples were inoculated with four seeds around the perimeter of the plate and one in the middle of the plate while maintaining a temperature of 121°C and an atmospheric pressure of 1 for 15 minutes. The plates were incubated for a stipulated period of 7 days at a temperature ranging from 25 to 37°C. After completion of the incubation period, the fungal isolates were purified by transferring a 5mm-diameter disk from each colony and planting it in another dish containing the same culture medium. The process was repeated several times to obtain pure isolates of the fungi. (17), (18), and the percentage of emergence of each fungus was calculated using the equation below.

$$\text{Appearance percentage} = \frac{\text{number of samples in which the fungus appeared}}{\text{The number of total samples}} \times 100$$

Preservation of fungal isolates

The fungal specimens were preserved in aseptic glass vials filled with potato dextrose agar media, positioned in an oblique orientation, and with circular pieces of the fungal material deposited on the growth medium in each vial. The specimens were thereafter subjected to incubation within a temperature range spanning from 25 to 37 °C for a duration of seven days, after which they were refrigerated until their utilization.

Testing Fungal Isolates for Aflatoxins

First: Ammonia Test

The aflatoxin-producing fungi were identified by growing them on previously prepared coconut medium, inoculating them with pure colonies produced on PDA medium at the age of seven days from isolated fungi, and incubating them in an incubator at a temperature of 25–27 °C for ten days. Following the emergence of mycelium, filter sheets were moistened with solution drops. Ammonia at a concentration of 20% was placed on the lid of the dishes in which the fungus grew, and the fungi producing toxins were distinguished from others by coloring the bases of the colonies red or orange instead of the transparent color (19).

Second: Detection of Aflatoxins Using Thin Layer Chromatography (TLC)

Fungal specimens were cultured on P.D.A. medium, whereby 5 mm-diameter discs of the fungi under study were centrally placed on each plate. The experiment was conducted in triplicate for each fungal isolate, with each petri dish incubated at a temperature ranging from 25 to 37 °C for a duration of one week. Subsequently, a single plate was chosen from each individual isolation. The culture medium on which the fungal isolate was cultivated was dissected into minute fragments using a sterile knife. Subsequently, the fragments were aseptically placed into an electric mixer containing 20 ml of chloroform using a sterile needle. The combination underwent blending for a duration of 10 minutes, followed by filtration using filter paper to separate the filtrate. (20) A line, characterized by its straightness and lightness, was inscribed on a thin-layer chromatography (TLC) plate, precisely at a distance of 1.5 cm from the base. Subsequently, a quantified volume of 15 microliters of AFB1 standard toxin was introduced onto the line at a position of 2 cm from the left edge of the plate and 2 cm from the original spot of the first standard toxin. The *Talaromyces* spp. extract samples were then placed on the same line, equidistant from the base, with an amount equal to that of the standard toxin. The same technique was applied to the remaining fungus samples, and the patches were left to desiccate. After this step, the patches were placed in a separation basin containing a 5:95 volume/volume combination of chloroform and methanol. The solution underwent monitoring until it attained a distance of approximately 2 cm from the upper extremity of the plate. Subsequently, the plates were extracted and subjected to a drying process for a duration of 5 minutes within the laboratory prior to being scrutinized under ultraviolet light at a wavelength of 360 nm. The purpose of this analysis was to detect the presence of aflatoxin B1, which was accomplished through a comparison of the migration coefficient, R_f, as well as the fluorescence color of the standard toxin (21).

Result

The Objective of This Study Is to Conduct Isolation and Identification of the Fungus *Talaromyces* Spp, Which Is Known to Be Associated With Local Grains.

In the present investigation, a total of 150 isolates pertaining to cystic fungi, specifically of the *Talaromyces* spp. type, were procured. This information is presented in Table 1.

Table 1: The number of isolates of the fungus *Talaromyces* spp. in stored grains

The incidence (%) of <i>Talaromyces</i> spp	Types of grain
95	Maize
90	Wheat
90	Barley
80	Field pistachio
75	Almonds
60	Cashews
55	Walnut

The findings of the field investigation conducted on the grains in the Karbala governorate have indicated the manifestation of *Talaromyces* spp., a type of fungus, in the majority of grain varieties and in varying degrees.



Figure 1: The ectodermal form of the fungus *Talaromyces columbinus* is grown on a medium PDA.

Detecting Aflatoxin B1 Production by Fungi Isolates

Various techniques were used to detect the ability of the studied fungus isolates to produce aflatoxins, namely

First: The Ammonia Method

The potential of various *Talaromyces* spp. isolates has been demonstrated through the utilization of coconut medium and ammonia in the detection assay, which were extracted from various grain types, to synthesize aflatoxins. This was substantiated by a marked alteration in the color of the colonies' bases, as well as the appearance of red to varying degrees. The color variation observed may be attributed to the isolates' varying capacities for generating aflatoxins, which is consistent with the claims made by (12) that the degree of red color is correlated with the quantity of aflatoxins produced. Based on the findings of the present study, the majority of fungi isolates possess the capability to produce aflatoxins, while a comparatively small proportion (5%) are unable to manufacture aflatoxins.

Second: Detection Using the Thin Plate Chromatography (T.L.C.) Method

Chemical detection by TLC showed the ability of fungal isolates to produce aflatoxin B1 based on the high fluorescence of the studied isolates. As shown in Figure No(2)

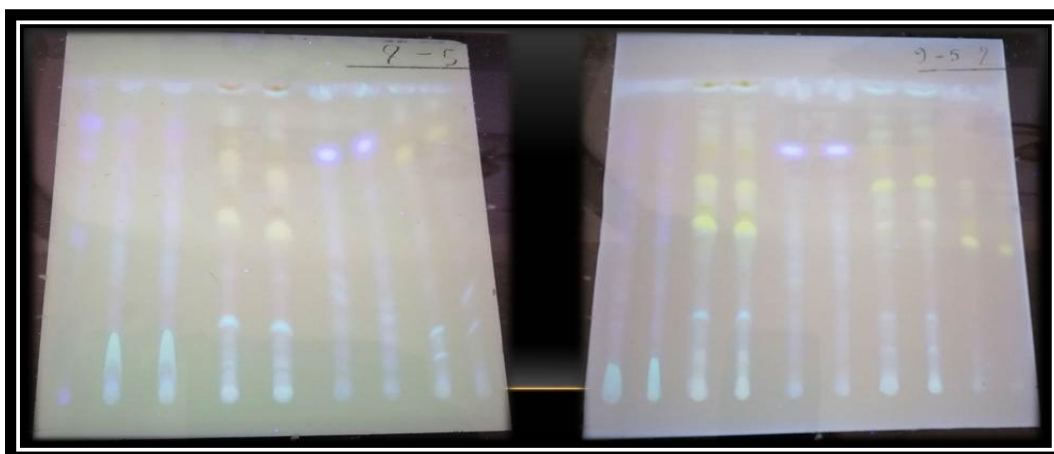


Figure 2: shows the species producing aflatoxin

The prevalence of aflatoxin-producing isolates was found to be as high as 98% in the majority of the grain species that were isolated for study. This worrying discovery emphasizes the significant risk that this toxin poses in grain consumption by people and animals, emphasizing the urgent need to reduce the risks of aflatoxin poisoning. These toxins are regarded as one of the most hazardous types of toxins known to date. This is due to the immune system's inability to identify them, leading to their widespread presence in various parts of the body. As a result, they cause immunodeficiency, renal impairment, liver destruction, and fetal deformity. Moreover, symptoms of poisoning may present themselves only after a considerable amount of time has passed, making it difficult to manage the situation. Therefore, controlling their spread is not feasible (22).

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