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Mycobiota Natural Occurrence of Aflatoxin in Some Egyptian Spices and Bio-Control by Essential Oils

Basma F. El Geoshi[1*]; M. A. El Metwally[2] and Magda I. Soliman[3]

[1]Mansoura Medical Research Center (MERC), Faculty of Medicine, Mansoura University.
[2]Mycological Research Department, Plant Pathology Research Institute, ARC, Giza.
[3]Botany Department, Faculty of Science, Mansoura University.

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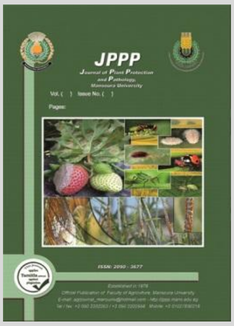

ABSTRACT

This survey has been carried out to determine, identify, and isolate fungi by standard blotter method (SBM), especially Aflatoxin-producing fungi; *Aspergillus* species that produce Aflatoxin B1, B2, G1, and G2 that are secondary metabolites, carcinogenic, and poisonous to humans and animals, from a total of 91 Egyptian spice samples (ginger, nutmeg, red chilli, and paprika) in Dakahlia markets and 11 hospital and medical centers in Mansoura. From all genera estimated, the genus *Aspergillus* had the highest frequency and prevalence, accounting for 291 of the total number of instances involving the isolation of fungi. Then *Pencillium* genus ranked second, accounting for 55, and *Fusarium* genus, accounting for 47. Also, 71 *Aspergillus flavus* group isolates were screened, and the aflatoxin concentrations of them were quantitatively calculated using HPLC. Moreover, isolates were determined to possess toxigenic properties, with their capacity to manufacture aflatoxins ranging from 15 to 521 ng/ml of culture filtrate. Molecular identification by ITS barcoding of the DNA of the studied strain was submitted to NCBI GenBank. The sequence length for the selected *Aspergillus* fungus was 457 bp, and the GC content was 58.2%. The inhibitory effect using ten essential oils with different concentrations was as follows: the most effective essential oils were clove oil, cinnamon oil and mint oil by inhibition ratio at 100% at 0.8% concentration for all of them.

Keywords: Aflatoxin; Spices; ITS ; Essential oils

INTRODUCTION

Throughout history, spices have held significant importance in the cultural practices and daily lives of individuals residing in specific regions. Throughout the ages, humans have employed herbs and spices both as culinary ingredients and for their medicinal properties. The presence of bio-molecules within plants is of utmost importance in the maintenance and promotion of overall health. Throughout history, spices have fulfilled many functions, encompassing their utilization as coloring agents, flavor enhancers, preservatives, food additives, and medicinal substances. Sachan *et al.* (2018). Spices possess many medical properties that find use in everyday life. Numerous spices commonly employed in culinary practices exhibit distinct medicinal activities, such as purgative, expectorant, carminative, and diuretic properties. Spices have been exploited for medical purposes since ancient times and continue to be utilized for such reasons in the present day. Sachan *et al.* (2018)

Spices and dried fragrant herbs found in stores have been found to carry a number of diseases. Worry and attention have increased as a result of the increased focus and knowledge regarding the safety of dried aromatic herbs and spices. To urge that FAO and WHO conduct a risk assessment on microbial dangers in these food items, the Codex Committee on Food Hygiene (CCFH) Organization (2022).

In addition to, spices may expose to microbial contamination in relation to pre- and post-harvest operations. Contamination has the potential to arise at various stages, including processing, storage, distribution, sale, and utilization. Sagoo *et al.* (2009). The usual practice of drying spices in open air leads to a significant presence of air- and soil-borne bacteria, fungus, and insects, hence resulting in a high level of contamination in most dried spices. Elbakhit (2019).

The contamination of mycotoxins is a prevalent occurrence on a global scale, resulting in a diverse range of adverse consequences and associated difficulties. Omotayo *et al.* (2019). They are dangerous spices contaminants, they display strong carcinogenicity especially Aflatoxins which recognized as teratogenic, mutagenic, carcinogenic agents leading to diseases like hepatic cellular carcinoma and liver cancer Sultan *et al.* (2024), and potent inhibitors of protein synthesis Omotayo *et al.* (2019). The ingestion of aflatoxin can lead to various symptoms such as nausea, vomiting, abdominal discomfort, convulsions, and other indications of acute hepatic impairment. Prolonged exposure to the substance also gives rise to a range of problems, such as stunted growth, cirrhosis, and hepatocellular cancer Dhakal *et al.* (2023). More than 18 different types of aflatoxins have been reported to date. Early estimation of fungal infection plays critical role in controlling of aflatoxin contamination. So, different methods, including culture, chromatographic techniques, and molecular assays, are used to detect aflatoxin contamination in crops and food products Shabeer *et al.* (2022).

Aflatoxins are hazardous secondary metabolites that are created by many toxigenic fungal species mainly *Aspergillus* species Sultan *et al.* (2024), which grow in soil, hay, decaying vegetation, grains and spices under certain

*** Corresponding author.**
E-mail address: drbasma.2010ss@gmail.com
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environmental conditions especially *Aspergillus flavus* and *Aspergillus parasiticus* which produce Aflatoxin B1, B2, G1 and G2 Dhakal *et al.* (2023).

Modifications to DNA sequences are highly advantageous for the purpose of developing distinctive markers that can be employed as a means of DNA barcoding across many species. A creative approach based on the diversity of nucleotide sequences is DNA barcoding. Ali *et al.* (2017). Because DNA barcoding is a reliable, quick, and affordable method, it is used for identification and offers additional, crucial support for morpho-species identification. The information from the standard portions of the genome's nucleotide sequences is what DNA barcodes rely on. Rayan (2019). The integration of DNA sequence comparisons within a species serves as a robust method for elucidating the evolutionary dynamics at play within certain gene areas. Additionally, it enables the recognition of pertinent factors pertaining to the evolutionary lineage of said species. Ramos-Onsins and Rozas (2002). The increased rate of nucleotide substitution, the relative ease of amplification, and the abundance of available sequencing data contribute to the significance of this phenomenon, the ITS regions has been considered as a very successful tool Eldemerdash *et al.* (2022).

There is an increasing tendency in the food and pharmaceutical sectors towards the utilization of medicinal herbs and spices Fakoor *et al.* (2013). Natural antimicrobial chemicals have a crucial role in food preservation and the management of microbial illnesses in humans and plants Baratta *et al.* (1998). Currently, a multitude of natural substances have been recognized as possessing antibacterial properties Nguefack *et al.* (2004), as essential oils are considered to be of greatest significance. These particular essential oils exhibit valuable antibacterial and antioxidant effects Fakoor *et al.* (2013).

The objective of this study is to examine and recovery the existence of Aflatoxin producing fungi *Aspergillus* species from Egyptian spices, DNA barcoding and molecular identification for studied isolates. Besides, Bio-controlling of Aflatoxin producing fungi by safe and inexpensive essential oils.

MATERIALS AND METHODS

Microbiological study:

Detection of spice fungi by seed health testing (SHT):

The detection of mycoflora present in 91 samples of spices (ginger , nut meg, paprika and red chili.) was conducted using the methodologies outlined by the International Seed Testing Association (ISTA) Association (1999) and the Standard Blotter Method (SBM) was employed to conduct the experiment.

The process of purifying and identifying of the seed-borne isolates of fungi by seed health testing:

By utilizing stereoscopic technology, binocular, hyphal tips from the colonized fungi on spices specimens were collected using capillary tubes, and transferred to plates having PDA media Dhingra and Sinclair (1996). The identification of the pure isolates was conducted through a process of comparing them with the description sheets of Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government institute of Seed Pathology (DGISP) Burgess *et al.* (1988) and Horn (1993).

Utilization of aflatoxins using high performance liquid chromatography (HPLC).

The sigma standards for aflatoxins, specifically AFB1, AFB2, AFG1, and AFG2, were employed throughout the duration of the current investigation. (Sigma, chemical company, USA). The established protocol for extraction, purification, and amount of aflatoxins using HPLC methodology. Int (2007). The High Performance Liquid Chromatography (HPLC) equipment utilized in this study was the Perkin-Elmer, series 200 system, manufactured in the United States.

Molecular study:

Molecular identification of four aflatoxins producing fungi

One Aflatoxin producing isolates were selected from the 71 isolates which have the highest value of AFB1 and molecular identification for this isolate were done as follow:

DNA extraction:

DNA extraction and purification for the studied isolate was carried out according DNeasy Tissue Kits (QIAGEN-Germany). The polymerase chain reaction amplification was conducted using a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems); primer sequence and product size in Table 1. The products of amplification underwent separation by electrophoresis. The PCR results were observed under ultraviolet light and captured through photography utilizing a Gel Documentation System (BIO-RAD 2000). The polymerase chain reaction products were sent to Faza Pazhouh Co. for sequencing using forward primers in an ABI 3730 xl DNA analyzer.

Sequence analysis;

The process of doing a sequence similarity search was carried out by utilizing the NCBI BLASTN online tool, which is accessible at http://ncbi.nlm.nih.gov/BLAST/. This search was conducted against the nucleotide collection (nr/nt) database. The default parameters for the BLASTN algorithm were utilized. A phylogenetic tree was generated by comparing the sequences of the ITS region. The length polymorphism of the PCR-amplified sequences and sequences from a database were used for this purpose. The tree was constructed using the blast tree construct method available at www.phylogeny.fr/simµle_phylogeny.cgi., and the estimation of GC contents was conducted for each sequence. The Neighbor Joining and Maximum Parsimony trees were generated using 1000 bootstrap repetitions. Felsenstein (1985).

Control of Aflatoxin by Different concentrations of plant essential oils:

Green tea, Caraway, Cinnamon, Mint, Black seed, Basil, Sage, Rose mary, Clove and Thyme were provided from the Department of Medicinal and Aromatic Plants, HRI, ARC, Egypt, air-dried, crushed, and hydrodistilled for three hours using a Clevenger apparatus Guenther (1972) to determine oil percentage in seeds. The heating source was Heating Mantle 2000 ml, FTHM-F500, Iksun-Dong, Jongro-Gu, Seoul, Korea (SCI FINETECH Co.).

Mycelium disks (0.5-cm diameter) from 7-days-old cultures Czapeck-Dox agar plate of *Aspergillus flavus* were used for inoculation 100 ml of yeast extract sucrose broth medium in 250 ml Erlenmayer flasks which were prepared and a suitable amounts of the tested 10 essential oils (Green tea, Caraway, Cinnamon, Mint, Black seed, Basil, Sage, Rose mary, Clove and Thyme) were added to the culture medium to give concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8(v/v%) Mostafa *et al.* (2011). After incubation period, the cultures were filtered and mycelial mats were collected on preweighed filter paper, dried in an oven at 70°C for 24 hrs. Mycelial dry weights were then determined Chang and Kim (2007).

The percentage of fungal growth inhibition was determined for each doses of essential oil using the following formula: The suppression percentage of fungal growth can be estimated by the next formula: [(Total weight of control - Total weight of sample) / Total weight of control] × 100.Chang and Kim (2007).

Table 1. The primers used for amplification of rDNA by PCR process Primer sequences and position of the primer sequence.

Primer Code	Sequence	Product Size	Position
(ITS-5) F	5′- GGAAGTAAAAGTCGTAACAAGG -3′	600-800bp	1737-1758
(ITS-4) R	5′- TCCTCCGCTTATTGATATGC-3′		2390-2409

RESULTS AND DISCUSSION

A large array of spices are frequently utilized as aromatics, colors, and flavors are imparted to meals by certain ingredients, commonly employed as appetizers to stimulate the appetite Jeswal and Kumar (2015). Moreover, significance of using spices and herbs in the preservation of food and their potential medicinal properties has been acknowledged Nguefack *et al.* (2004). Similar to numerous a varieties of agricultural commodities, spices and herbs are susceptible to various forms of being contaminated by microorganisms during both of pre- and post-harvest stages. Also, infection by microbes has the potential to arise during various stages, including the process of handling, storing, distributing, and sale and utilization. McKee (1995).

The predominant microorganisms that commonly contaminate spices are fungi as the mycological quality of many spices available on the market exhibits significant deficiencies, as evidenced by the presence of several genera and species of fungi Jeswal and Kumar (2015). Fungi are a prevalent constituent of the microflora found in food and can potentially contribute to food degradation and the generation of mycotoxins. The presence of aflatoxins in spices can lead to potential carcinogenic effects, even when ingested in minimal quantities McKee (1995). The majority of mycotoxins are synthesized by fungal species classified under the genera *Aspergillus*, *Penicillium*, and *Fusarium*. These genera, namely *Aspergillus* and *Penicillium*, are usually referred to as storage molds Romagnoli *et al.* (2007).

In this study, there were variations presented in the spices mycoflora isolated from different types of investigated spices. Twenty three fungal species belonging to 11 genera were observed and identified. The species that is most commonly observed and widely distributed in the genus *Aspergillus* was *Aspergillus niger.*

A total of 91 samples were collected, encompassing 4 distinct spices sourced from 11 diverse districts. in Dakahlia as 2 samples were collected. The districts were Aga, Bilkas, Dekrnis, El Gamalia, Gamasa, Mansoura, Met Ghamr, Nabaro, Sherben, Snbelawin and Talkha. Twenty three fungal species belonging to 11 genera were observed and subsequently identified i.e., *Acremoniella, Alternaria, Aspergillus, Cephalosporum, Chaetomium, Emericella, Fusarium, Penicillium, Phoma, Rhizopus* and *Trichoderma.* Both of *Aspergillus* and *Penicillium* were the predominant genera. Eleven *Aspergillus* species, including: *Aspergillus flavipes, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucaus, Aspergillus niger, Aspergillus nomius, Aspergillus ochraecous, Aspergillus oryzae, Aspergillus parasiticus, Aspergillus tamari* and *Aspergillus terreus* were identified. The most frequently occurring and widespread species in the genus *Aspergillus* was *Aspergillus niger* Table 2 and 7.

The same were mentioned by Romagnoli *et al.* (2007), who said that spices have been found to provide as a favorable substrate for the colonization of various species of *Aspergillus*, *Penicillium*, and *Fusarium*. Additional fungal species that were present in limited quantities were *Trichoderma*, Pestalotia, *Rhizopus*, *Cladosporium*, and a few actinomycetes. Also, Hammami *et al.* (2014) found that among the spices that were tested, it was found that *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger, Aspergillus ustus, Cladosporium cladosporidies, Curvularia lunata, Fusarium oxysporum, Fusarium roseum, Helminthosporium tetramera*, and *Trichoderma viride* exhibited the highest occurrence rate on the agar plate. Additionally, *Alternaria alternata, Aspergillus flavus, Aspergillus niger*, and *Helminthosporium tetramera* demonstrated the maximum incidence on the blotter plate.

Table 3 and Figure 1 demonstrated that the most prevalent fungus was *Aspergillus niger* encounting 709.94 then *Aspergillus parasiticus* of total count 416.65in all Ginger samples. Not only that but also, the prevalent center with fungal distribution was Nabro2 sample by total fungal count 266.60. The most ranked fungus was *Rhizopus sp*, *Aspergillus niger* and *Aspergillus parasiticus* in all studied samples and that was in agreement with Hashem and Alamri (2010) demonstrated that *Aspergillus niger* has been identified as the *Aspergillus* species with the highest frequency, then *Aspergillus flavus.*Nguefack et al. (2004) Moreover, data demonstrated that the most prevalent fungus was *Aspergillus niger* then *Aspergillus parasiticus* in all ginger samples and Elshafie *et al.* (2002) reported that A total of 20 species of fungi were identified, including *Aspergillus flavus* and *Aspergillus niger* Elshafie *et al.* (2002). Furthermore, *Penicillium* and *Rhizopus* had the highest prevalence among a total of 105 samples collected from seven different spices, namely cumin, cinnamon, clove, black pepper, cardamom, ginger, and coriander El Mahgubi *et al.* (2013).

The result of Nut meg samples presented in Table 4 and Figure 1 indicated that the most frequent fungus was

Aspergillus niger encounting 1193.27 then *Aspergillus flavipes* of total count 146.61 in all investigated samples. Moreover, samples of Nut meg showed that the prevalent center with fungal load was Aga1 sample by total fungal count 166.5. Samples of Nut meg indicated that the most frequent fungus was *Aspergillus niger* then *Aspergillus flavipes*. Also, ARIFAH *et al.* (2023) said the fungal species *Lasiodiplodia theobromae*, *Aspergillus niger*, and *Aspergillus flavus* had the highest frequency in the nutmeg kernels. *Aspergillus niger* was shown to be the predominant spoilage fungus isolated from *Myristica fragrans* seeds, exhibiting the largest population. The present discovery suggests that *Aspergillus niger* can be classified as a type of deterioration fungi that has been detected from *Myristica fragrans*. Fendiyanto *et al.* (2021).

The detected fungi in Paprika samples in Table 5 & Figure 2, showed that the most frequent fungus was *Aspergillus niger* encounting 1019.92 then *Rhizopus sp* of total count 823.29 and ranked in third *Aspergillus flavus* by fungal population 283.28 in all Paprika samples. In addition to, samples of interest indicated that the highest fungal population predominant center was Snbelwin1 sample by total fungal count 303.1. Also, data of fungi in Red chilli samples in Table 6 & Figure 2 determined that the most present fungus was *Aspergillus niger* counting 1603.26 then *Rhizopus sp* by total count 1197.31 in all studied samples. In addition to, the predominant center with fungal load was Met Ghamr1 sample by total fungal count 423.2. Furthermore, the result revealed that the most present fungus was *Aspergillus niger* then *Rhizopus sp* and *Aspergillus flavus* in red chili and paprika samples. Mandeel (2005), It was observed that among the 17 spices that were examined, chillies exhibited the most significant fungal contamination resulting from the existence of *Aspergillus flavus* and *Aspergillus niger* McKee (1995). Also, El Mahgubi *et al*. (2013) said that *Aspergillus* was the most prevalent one in paprika samples, the sections Nigri and Flavi exhibited the highest prevalence.

From all genera estimated, the genus *Aspergillus* had the highest frequency and prevalence encounting 291 of total count of isolated cases of fungi then *Pencillium* genus ranked in second encounting 55 of total number of incidences related to the isolation of fungi in all spices samples as shown in Table 7. Furthermore, ranked in third *Fusarium* genus encounting 47 of overall count of instances of isolation. of fungi in all spices samples. Also, the most frequent fungus in *Aspergillus* group were *Aspergillus niger* and *Aspergillus flavus* by total number of cases of isolation 86, 49 respectively.

The result detected that the most frequent fungus was *Aspergillus flavus* and *Aspergillus niger* in studied samples and that was the same as El Mahgubi *et al.* (2013) who said that the isolates of *Aspergillus* that obtained from spices are classified into 9 distinct. The sections Nigri and Flavi exhibited the highest prevalence. Also, obtained data showed that the most prevalent fungus was *Aspergillus niger* then *Rhizopus sp* in all samples. The same was revealed by Koutsias *et al*. (2021) who said that the initial occurrence of contamination in the investigated samples is detected to be caused by *Aspergillus* spp., with the predominant presence being attributed to aflatoxigenic *Aspergillus* spp. and *Rhizopus spp*. were identified to be in the second dominant fungi found in samples of coriander, cardamom, caraway, ginger, and fenugreek.

The given information in Table 7 demonastrated that *Aspergillus niger* was the highest of relative density as the count of isolation of *A niger* in the total number of all fungi isolated was 121.12%, then *Pencillium* sp by relative density ratio 77.46% followed by *Rhizopus* sp yielding 63.38% of relative density. While, the lowest number of isolation of fungus compared to all fungi isolated were for *Alternaria* sp, *Cephalosporum* sp. and *Trichoderma* sp. by ratio 2.81% for each one of them then *Phoma* sp by ratio 4.22%. Moreover, as apparent in Table 6 the highest and prevalent frequency of occurrence was *Aspergillus niger, Pencillium* sp *Aspergillus flavus* and *Rhizopus* sp, encountering 94.50%, 60.43% 53.84% and 49.45% respectively. On the other hand, *Alternaria* sp, *Cephalosporum* sp. and *Trichoderma* sp. were isolated in rare frequency of occurrence by ratio 2.19% of all the samples.

From all genera estimated, the genus *Aspergillus* had the highest frequency and prevalence. and that was in agreement with Azzoune *et al.* (2016) who said that the fungi that were often isolated in this study included the identified genera of fungi in the sample consisted of *Aspergillus* (56.4%), *Penicillium* (25.1%), *Mucor* (12.8%), and *Eurotium* (5.7%). The Flavi section of the *Aspergillus* genus included 28.9% of the total *Aspergilli* species. and Bokhari (2007) found that the genera that exhibited the highest prevalence were *Aspergillus*, *Penicillium*, and *Fusarium*. Bokhari (2007). Also, the most frequent fungus were *Aspergillus niger* and *Aspergillus flavus* and that were in agreement with Migahed *et al.* (2017) who detected that stated that the fungi most commonly identified in the samples from different spices were *Aspergillus flavus, Aspergillus niger,* and *Aspergillus parasiticus.* Then *Pencillium* genus ranked in second.

It was obvious, that *Aspergillus niger* was the highest of relative density then *Rhizopus* sp followed by *Pencillium*. Prevalent frequency of occurrence was *Aspergillus niger, Pencillium* sp *Aspergillus flavus* and *Rhizopus* sp. The same mentioned by Migahed *et al.* (2017) who found that *Aspergillus* was the highest frequency and most genus of prevalence then *Penicillium* was the second highest prevalence genus and *Rhizopus* was ranked third detected in considerable rate of presence of all studied spices. Furthermore, Hashem and Alamri (2010) who detected that the fungal genera that were most commonly observed were *Aspergillus*, *Penicillium*, and *Rhizopus*.

Table 2. List of identified seed borne fungi of different spices by seed health testing

***Acremoniella* sp**	***Aspergillus oryzae***	***Fusarium solani***
Alternaria sp	*Aspergillus parasiticus*	*Fusarium oxysporum*
Aspergillus flavipes	*Aspergillus tamari*	*Penicillum sp. Thom*
Aspergillus flavus	*Aspergillus terrous*	*Phoma* sp
Aspergillus fumgiatus	*Cephalosporium* sp	*Rhizopus* sp
Aspergillus glaucus	*Chaetomium* sp	*Trichoderma* sp.
Aspergillus niger	*Emercella nomius*	
Aspergillus nomius	*Fusarium moniliforme*	
Aspergillus ochraceus		

Table 3. Incidence of seed-borne fungi in *Zingiber officinale* (Ginger) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumgiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Aga 1	0.0	0.0	0.0	0.0	0.0	0.0	20 ± 10	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	3	2	29.9
Aga 2	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	23.33± 20.81	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	3	2	33.2
Bilkas 1	0.0	0.0	0.0	0.0	0.0	0.0	40 ± 36.05	3.33 ± 5.77	0.0	0.0	53.33± 20.81	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.66± 15.27	0.0	0.0	0.0	4	2	113.2
Bilkas 2	0.0	0.0	0.0	9.33 ± 6.11	0.0	0.0	13.33± 8.32	0.0	5.33 ± 6.11	0.0	0.0	5.33 ± 2.30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.33 ± 2.30	0.0	16 ± 27.71	0.0	6	3	50.5
Dekmis 1	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	3	3	16.5
Dekmis 2	46.66 ± 20.81	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	4	4	56.5
El Gamalia	0.0	0.0	0.0	13.33± 5.77	0.0	0.0	23.33± 5.77	0.0	0.0	0.0	0.0	13.33± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.33± 11.54	0.0	0.0	0.0	4	2	63.2
Gamasa 1	0.0	0.0	0.0	3.33 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1	1	3.3
Gamasa 2	0.0	0.0	0.0	6.66 ±5.77	0.0	0.0	20 ± 10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	3	2	29.9
Mansoura 1	0.0	0.0	0.0	63.33 ±5.77	0.0	0.0	50 ±26.45	0.0	0.0	0.0	0.0	0.0	43.33± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	43.33 ±15.27	0.0	6.66 ±11.54	0.0	5	3	206.5
Mansoura 2	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ±11.54	6.66 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	46.66 ±41.63	0.0	3	2	59.5
Met Ghamr 1	0.0	0.0	0.0	0.0	0.0	0.0	23.33± 5.77	0.0	0.0	0.0	10±17.32	0.0	0.0	0.0	0.0	0.0	0.0	23.33± 5.77	0.0	10 ± 10	0.0	0.0	0.0	4	3	66.6
Met Ghamr2	0.0	0.0	0.0	0.0	0.0	0.0	26.66± 15.27	3.33 ±5.77	0.0	0.0	0.0	13.33 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.33 ±5.77	0.0	23.33 ±5.77	0.0	5	3	79.8
Nabaro 1	3.33 ±5.77	0.0	0.0	0.0	0.0	0.0	3.33 ±5.77	0.0	0.0	26.66± 15.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ±5.77	0.0	6.66 ±11.54	0.0	5	4	43.1
Nabaro 2	0.0	0.0	0.0	0.0	0.0	0.0	83.33± 15.27	0.0	3.33 ±5.77	0.0	100 ±0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40 ±26.45	0.0	40 ±10	0.0	5	3	266.6

Cont. 3. Incidence of seed-borne fungi in *Zingiber officinale* (Ginger) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumgiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Sherben 1	0.0	0.0	0.0	53.33 ± 41.63	0.0	0.0	33.33 ± 28.86	6.66 ± 11.54	10 ± 10	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	6	2	116.4
Sherben 2	0.0	0.0	0.0	10 ± 10	0.0	0.0	20 ± 10	3.33 ± 5.77	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	5	2	39.9
Snbelawin 1	13.33 ± 15.27	0.0	0.0	0.0	0.0	0.0	83.33 ± 5.77	0.0	0.0	0.0	50 ± 26.45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40 ± 10	0.0	0.0	0.0	4	3	186.6
Snbelawin 2	0.0	0.0	0.0	26.66 ± 5.77	0.0	0.0	46.66 ± 15.27	6.66 ± 5.77	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	13.33 ± 11.54	0.0	36.66 ± 5.77	0.0	0.0	0.0	6	3	133
Talkha 1	0.0	0.0	0.0	0.0	0.0	0.0	73.33 ± 20.81	6.66± 5.77	13.33 ± 23.09	0.0	93.33 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	3.33± 5.77	0.0	30 ± 10	0.0	0.0	0.0	6	3	219.83
Talkha 2	0.0	0.0	0.0	0.0	0.0	0.0	73.33 ± 5.77	6.66 ± 5.77	13.33 ± 11.54	0.0	83.33 ± 5.77	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	13.33 ± 11.54	0.0	30 ± 17.32	0.0	0.0	0.0	7	3	223.1
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20 ± 26.45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	2	2	23.3
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
6	0.0	0.0	0.0	0.0	0.0	0.0	46.66 ± 50.33	50 ± 43.58	10 ± 17.32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	4	2	109.9
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1	1	6.6
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
Total count	63.26	6.66	0.0	192.63	0.0	0.0	709.94	93.29	65.31	33.32	416.65	48.64	43.33	0.0	0.0	0.0	0.0	53.32	3.33	311.27	0.0	139.31	0.0			

1.Mansoura University City.
2. Gastroenterology Hospital, The emergency hospital and Student Hospital.
3. Mansoura Specialized Hospital, Ophthalmology Hospital and Talkha Hospital.
4. Specialized Medical Hospital.
5. Ophthalmic Center.
6. Childern's Hospital Mansoura University.
7. Mansoura University Hospital.
8. Oncology center Mansoura University.
9. Mansoura Chest Disease Hospital and New Mansoura General Hospital.

Table 4. Incidence of seed-borne fungi in *Myristica fragrans* (Nut meg) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumigiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella. nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Aga 1	0.0	0.0	0.0	0.0	0.0	0.0	96.66 ± 5.77	36.66 ± 15.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10 ± 10	0.0	3.33 ± 5.77	0.0	20 ± 34.64	0.0	5	4	166.5
Aga 2	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	16.66 ± 15.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	3.33 ± 5.77	0.0	0.0	0.0	4	3	29.8
Bilkas 1	0.0	0.0	6.66 ± 11.54	6.66 ± 11.54	0.0	0.0	30 ± 10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20 ± 10	0.0	3.33 ± 5.77	0.0	10 ± 10	0.0	0.0	0.0	6	4	76.5
Bilkas 2	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	66.66 ± 5.77	0.0	10 ± 10	0.0	0.0	0.0	0.0	0.0	0.0	33.33 ± 5.77	0.0	0.0	0.0	16.66 ± 15.27	0.0	0.0	6.66 ± 5.77	6	4	136.4
Dekrnis 1	0.0	0.0	0.0	3.33± 5.77	0.0	0.0	100± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	1	103.3
Dekrnis 2	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	50 ± 20	0.0	3.33 ± 5.77	0.0	0.0	0.0	10 ± 17.32	0.0	0.0	0.0	0.0	20 ± 10	0.0	0.0	0.0	0.0	0.0	5	2	89.9
El Gamalia	0.0	0.0	10 ± 0	10 ± 10	0.0	6.66 ± 5.77	46.66 ± 15.27	3.33 ± 5.77	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20 ± 10	0.0	6.66 ± 5.77	0.0	0.0	0.0	8	3	106.4
Gamasa 1	0.0	0.0	16.66 ± 11.545	6.66 ± 5.77	0.0	0.0	53.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	5	2	89.7
Gamasa 2	0.0	0.0	6.66± 5.77	10 ±0	0.0	0.0	40± 10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33± 5.77	0.0	0.0	0.0	4	2	59.9
Mansoura 1	0.0	0.0	50±1 7.32	3.33± 5.77	0.0	3.33± 5.77	50± 17.32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.33 ±5.77	0.0	0.0	0.0	5	2	119.9
Mansoura 2	0.0	0.0	0.0	0.0	0.0	6.66± 5.77	50±2 6.45	0.0	0.0	0.0	10±0	0.0	13.33 ±5.77	0.0	0.0	0.0	0.0	10±1 0	0.0	10±0	0.0	6.66± 11.54	0.0	7	4	106.5

Cont. 4. Incidence of seed-borne fungi in *Myristica fragrans* (Nut meg) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumigiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella. nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Met Ghamr 1	0.0	0.0	3.33 ± 5.77	0.0	0.0	6.66 ± 5.77	76.66 ± 15.27	3.33 ± 5.77	3.33 ± 5.77	0.0	16.66 ± 15.27	0.0	13.33 ± 5.77	0.0	0.0	0.0	0.0	23.33 ± 15.27	0.0	3.33 ± 5.77	0.0	0.0	0.0	9	3	149.6
Met Ghamr 2	0.0	0.0	3.33 ± 5.77	10 ± 10	0.0	3.33 ± 5.77	50 ± 10	0.0	3.33 ± 5.77	0.0	0.0	0.0	10 ± 0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	6.66 ± 5.77	0.0	0.0	0.0	8	3	93.1
Nabaro 1	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	33.33 ± 20.81	3.33 ± 5.77	0.0	0.0	13.33 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	16.66 ± 28.86	0.0	0.0	0.0	0.0	0.0	5	2	69.8
Nabaro 2	0.0	0.0	13.33 ± 11.54	6.66 ± 5.77	0.0	0.0	53.33 ± 23.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.33 ± 15.27	0.0	3.33 ±5 .77	0.0	0.0	0.0	5	3	99.8
Sherben 1	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	46.66 ± 15.27	0.0	0.0	0.0	3.33 ± 5.77	0.0	13.33 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	5	2	69.8
Sherben 2	0.0	0.0	3.33± 5.77	6.66± 5.77	0.0	0.0	43.33 ±5.77	0.0	0.0	0.0	0.0	0.0	10±0	0.0	0.0	0.0	0.0	0.0	0.0	3.33± 5.77	0.0	0.0	0.0	5	2	66.5
Srbelawin 1	0.0	0.0	3.33 ± 5.77	10 ± 0	0.0	0.0	53.33 ± 30.55	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	10 ± 17.32	0.0	6	3	86.5
Srbelawin 2	0.0	0.0	13.33 ± 11.54	10 ± 10	0.0	0.0	83.33 ± 15.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	4	2	113.2
Talkha 1	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	53.33 ± 37.85	16.66 ± 11.54	10 ± 17.32	0.0	0.0	0.0	23.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	20 ± 10	0.0	6.66 ±1 1.54	0.0	7	3	133.1
Talkha 2	0.0	0.0	0.0	0.0	0.0	0.0	100 ± 0	0.0	0.0	0.0	23.33 ± 15.27	3.33 ± 5.77	16.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	16.66 ± 11.54	0.0	6.66 ± 5.77	0.0	6	3	166.4
Specie count	0.0	0.0	146.61	89.96	0.0	29.97	1193.27	63.31	36.65	0.0	66.65	3.33	116.64	0.0	0.0	53.33	0.0	136.64	0.0	143.26	0.0	49.98	6.66			

Table 5. Incidence of seed-borne fungi in *Capsicum annuum* (Paprika) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumgiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Aga 1	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	96.66 ± 5.77	0.0	0.0	0.0	30 ± 20	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	53.33 ± 23.09	0.0	5	4	189.8
Aga 2	23.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	33.33 ± 5.77	0.0	0.0	0.0	13.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	16.66 ± 11.54	0.0	0.0	0.0	0.0	80 ± 17.32	0.0	5	4	166.5
Bilkas 1	13.33 ± 11.54	0.0	0.0	13.33 ± 11.54	0.0	0.0	20 ± 10	10 ± 17.32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	5	3	63.2
Bilkas 2	0.0	0.0	10 ± 10	13.33 ± 11.54	0.0	0.0	33.33 ± 20.81	6.66 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.66 ± 15.27	30 ± 30	0.0	0.0	0.0	36.66 ± 5.77	0.0	7	3	146.4
Dekrnis 1	0.0	0.0	0.0	0.0	0.0	0.0	70 ±10	0.0	0.0	100 ±0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	1	170
Dekrnis 2	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	50 ± 26.45	13.33 ± 5.77	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	13.33 ± 23.09	0.0	6	3	89.8
El Gamalia	0.0	0.0	3.33 ± 5.77	40 ± 10	0.0	0	36.66 ± 20.81	3.33 ± 5.77	30 ± 26.45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10 ± 17.32	0.0	100 ± 0	6.66 ± 11.54	8	3	229.8
Gamasa 1	0.0	0.0	0.0	0.0	0.0	0.0	23.33 ± 11.54	13.33 ± 11.54	0.0	0.0	16.66 ± 20.81	0.0	0.0	0.0	16.66 ± 20.81	0.0	0.0	0.0	0.0	0.0	0.0	33.33 ±5 .77	0.0	5	3	103.1
Gamasa 2	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	13.33 ± 5.77	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	0.0	3.33 ± 5.77	0.0	23.33 ± 5.77	0.0	6	4	59.7
Mansoura 1	0.0	0.0	3.33 ± 5.77	73.33 ± 25.16	0.0	0.0	100 ± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.66 ± 11.54	0.0	0.0	0.0	0.0	100 ± 0	0.0	5	3	293.2
Mansoura 2	0.0	0.0	0.0	10 ± 10	0.0	0.0	23.33 ± 5.77	20 ± 10	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	20 ± 17.32	0.0	6.66 ± 11.54	3.33 ± 5.77	0.0	33.33 ± 5.77	0.0	8	5	123.1
Met Ghamr 1	0.0	0.0	0.0	16.66 ± 5.77	0.0	10 ± 10	66.66 ± 15.27	20 ± 10	6.66 ± 11.54	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10 ± 0	10 ± 0	0.0	3.33 ± 5.77	0.0	100 ± 0	0.0	9	4	243.1
Met Ghamr 2	0.0	0.0	3.33 ± 5.77	0.0	0.0	6.66 ± 5.77	66.66 ± 23.09	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10 ± 10	0.0	23.33 ± 5.77	0.0	6	3	113.1
Nabaro 1	26.66 ± 5.77	0.0	0.0	3.33 ± 5.77	0.0	0.0	10 ± 10	6.66 ± 11.54	0.0	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	23.33 ± 5.77	20 ± 10	0.0	0.0	0.0	23.33 ± 5.77	0.0	8	4	119.7
Nabaro 2	43.33 ± 20.81	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	13.33 ± 5.77	0.0	0.0	40 ± 20	0.0	0.0	0.0	0.0	0.0	20 ± 10	10 ± 0	6.66	0.0	0.0	0.0	0.0	6	3	133.2

Cont. 5. Incidence of seed-borne fungi in *Capsicum annuum* (Paprika) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumgiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Sherben1	0.0	0.0	0.0	6.66± 5.77	0.0	0.0	26.66 ±5.77	6.66± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20 ±10	0.0	0.0	0.0	0.0	23.33 ±5.77	0.0	5	3	83.1
Sherben2	0.0	0.0	0.0	6.66 ±5.77	0.0	0.0	16.66 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ±5.77	0.0	0.0	20 ±10	0.0	4	3	49.8
Snbelawin 1	6.66 ± 5.77	0.0	0.0	0.0	0.0	0.0	96.66 ± 5.77	0.0	0.0	0.0	53.33 ± 11.54	0.0	0.0	0.0	0.0	0.0	23.33 ± 5.77	100 ± 0	0.0	0.0	0.0	23.33 ± 5.77	0.0	6	4	303.1
Snbelawin 2	26.66 ± 15.27	0.0	0.0	33.33 ± 35.11	0.0	0.0	36.66 ± 35.11	26.66 ± 30.55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	30 ± 0	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	6	4	156.4
Talkha1	0.0	0.0	3.33 ± 5.77	46.66 ± 41.63	0.0	0.0	70 ± 17.32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33 ± 5.77	6.66 ± 5.77	3.33 ± 5.77	20 ± 17.32	0.0	7	5	153.1
Talkha2	0.0	0.0	0.0	10 ±10	0.0	0.0	23.33 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66± 5.77	3.33± 5.77	23.33 ±5.77	0.0	5	4	66.5
1	0.0	0.0	0.0	0.0	0.0	0.0	100 ± 0	16.66 ± 15.27	0.0	0.0	70 ± 17.32	0.0	0.0	16.66 ± 20.81	0.0	0.0	0.0	0.0	0.0	0.0	0.0	93.33 ± 11.54	0.0	5	3	296.5
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
Total count	146.63	0.0	23.32	283.28	0.0	16.66	1019.92	163.28	39.99	106.66	229.98	0.0	0.0	16.66	29.98	0.0	206.63	170.0	16.65	49.97	6.66	823.29	6.66			

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Table 6. Incidence of seed-borne fungi in *Capsicum frutescene* (Red chilli) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumgiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Aga 1	0.0	0.0	0.0	0.0	0.0	0.0	100±0	0.0	0.0	100±0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10±10	0.0	3.33±5.77	0.0	100±0	0.0	5	4	113.3
Aga 2	0.0	0.0	0.0	13.33 ± 23.09	0.0	0.0	50 ± 10	20 ± 10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	1	83.3
Bilkas1	0.0	0.0	0.0	0.0	0.0	0.0	26.66 ±5.77	0.0	0.0	0.0	13.33 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.66 ±5.77	0.0	3	2	66.5
Bilkas2	0.0	0.0	0.0	23.33 ± 5.77	0.0	0.0	66.66 ± 15.27	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	10 ± 0	0.0	0.0	0.0	26.66 ± 5.77	0.0	6	4	136.4
Dekrnis 1	0.0	0.0	0.0	3.33 ± 5.77	0.0	0.0	16.66 ± 15.27	0.0	0.0	0.0	56.66 ± 15.27	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	10 ± 0	0.0	0.0	0.0	5	3	89.8
Dekrnis 2	0.0	0.0	0.0	0.0	0.0	0.0	100 ± 0	0.0	2.66 ± 4.61	44.33 ± 12.66	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.66 ± 17.21	0.0	0.0	14 ± 12.76	0.0	5	3	174.5
ElGamalia	0.0	0.0	0.0	0.0	0.0	3.33± 5.77	96.66 ±5.77	0.0	96.66 ±5.77	0.0	96.66 ±5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100± 0	0.0	5	2	393.1
Gamasa 1	0.0	0.0	0.0	0.0	0.0	0.0	46.66 ± 30.55	0.0	3.33 ± 5.77	100 ± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20 ± 34.64	0.0	0.0	0.0	76.66 ± 5.7	0.0	5	3	246.5
Gamasa 2	0.0	0.0	0.0	13.33 ± 5.77	0.0	0.0	26.66 ± 5.77	63.33 ± 20.81	6.66 ± 5.77	0.0	0.0	10 ± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5	1	119.8
Mansoura 1	0.0	0.0	0.0	100 ± 0	0.0	0.0	100 ± 0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10 ± 10	0.0	0.0	0.0	90 ± 10	0.0	5	3	303.3
Mansoura 2	0.0	0.0	0.0	6.66± 5.77	0.0	0.0	100± 0	0.0	3.33± 5.77	100± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100± 0	0.0	5	2	309.9
Met Ghamr 1	0.0	0.0	0.0	100 ± 0	0.0	0.00	100 ± 0	0.0	83.33 ± 20.81	0.0	0.0	26.66 ± 23.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.33 ± 11.54	0.0	100 ± 0	0.0	6	3	423.2
Met Ghamr 2	0.0	0.0	0.0	36.66 ± 15.27	0.0	0.0	56.66 ± 25.16	0.0	0.0	0.0	0.0	13.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66 ± 5.77	0.0	0.0	0.0	4	2	113.1
Nabaro 1	0.0	0.0	0.0	100± 0	40±1 0	0.0	100± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.33 ±5.77	0.0	0.0	0.0	4	2	263.3
Nabaro 2	0.0	0.0	0.0	0.0	3.33± 5.77	0.0	80±1 0	100± 0	6.66± 5.77	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	3.33± 5.77	0.0	0.0	0.0	100± 0	0.0	6	3	293.2

Cont. 6. Incidence of seed-borne fungi in *Capsicum frutescene* (Red chilli) samples in Dakahlia, using seed health test.

Fungi / Sites	*Acremoniella sp*	*Alternaria sp*	*A flavipes*	*A flavus*	*A fumgiatus*	*A glaucus*	*A niger*	*A. nomius*	*A ochraceus*	*A oryzae*	*A parasiticus*	*A tamarii*	*A terrous*	*Cephalosporum*	*Chaetomium sp*	*Emercella. nomius*	*F moniliform*	*F solani*	*F oxysporum*	*Penicillum sp.*	*Phoma sp*	*Rhizopus sp*	*Trichoderma sp.*	*No. of Species*	*No. of Genera*	*Total count*
Sherben 1	0.0	0.0	0.0	0.0	0.0	0.0	93.33 ± 5.77	0.0	16.66 ± 11.54	100 ± 0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	10 ± 10	0.0	0.0	0.0	50 ± 10	0.0	6	3	273.2
Sherben 2	0.0	0.0	0.0	30 ± 10	0.0	0.0	36.66 ± 5.77	0.0	6.66 ± 5.77	0.0	0.0	3.33 ± 5.77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.33 ± 15.27	0.0	5	2	109.8
Snbelawin 1	0.0	0.0	0.0	0.0	6.66± 5.77	0.0	100± 0	0.0	3.33± 5.77	100 ±0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66± 11.54	0.0	0.0	0.0	80 ±10	0.0	6	3	296.5
Snbelawin 2	0.0	0.0	0.0	0.0	6.66 ± 11.54	0.0	36.66 ± 20.81	30 ± 43.58	0.0	0.0	0.0	0.0	0.0	0.0	40 ± 10	0.0	0.0	0.0	13.33 ± 15.27	0.0	0.0	0.0	0.0	5	3	126.5
Talkha 1	0.0	0.0	0.0	90± 10	0.0	0.0	10± 10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.66± 40.41	13.33± 15.27	0.0	10± 10	0.0	0.0	0.0	0.0	0.0	5	4	159.9
Talkha 2	0.0	0.0	0.0	33.33± 15.27	0.0	0.0	6.66± 5.77	0.0	6.66± 11.54	0.0	0.0	0.0	0.0	0.0	20±0	16.66± 15.27	0.0	10±1 0	0.0	10±1 0	26.66± 46.18	0.0	0.0	8	6	129.7
1	0.0	0.0	0.0	0.0	0.0	0.0	100± 0	100±0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100± 0	0.0	3	2	300
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
6	0.0	0.0	0.0	0.0	0.0	0.0	70 ±0	6.66± 11.54	53.33± 11.54	63.33± 15.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.66± 5.77	0.0	13.33± 11.54	0.0	100± 0	0.0	7	4	313.1
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
8	0.0	0.0	0.0	6.66± 5.77	0.0	0.0	83.33± 15.27	100 ±0	100± 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.33 ±5.77	0.0	100± 0	0.0	6	3	403.2
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
Total count	0.0	0.0	0.0	566.63	56.65	3.33	1603.26	419.99	395.93	607.66	166.65	56.65	0.0	3.33	103.32	29.99	0.0	89.99	33.65	93.31	26.66	1197.31	0.0			

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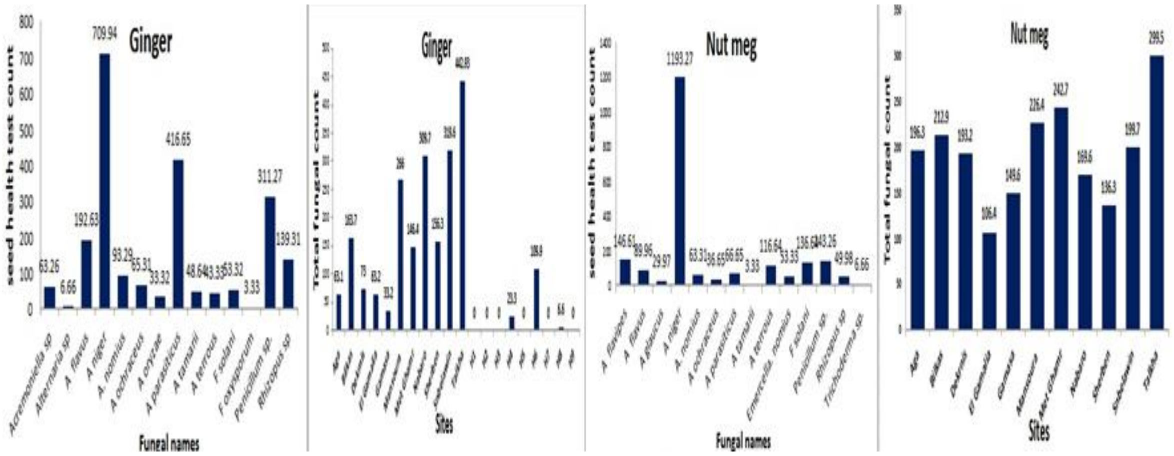

Figure 1. Prevalence of seed-borne fungi in *Zingiber officinale* (Ginger) and *Myristica fragrans* (Nut meg) samples in Dakahlia, using seed health test.

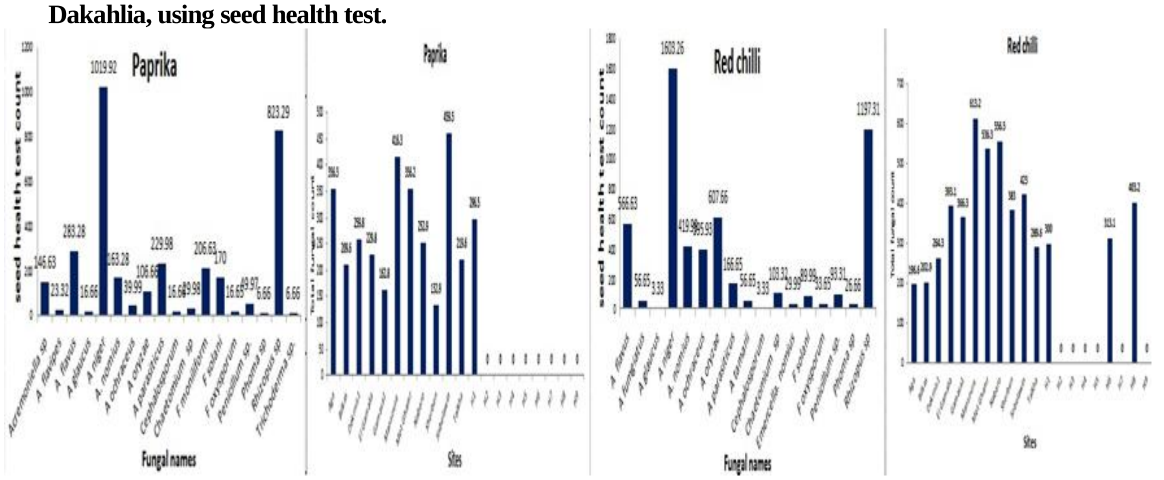

Figure 2. Prevalence of seed-borne fungi in Capsicum *annuum* (Paprika) and *Capsicum frutescene* (Red chilli) samples in Dakahlia, using seed health test.

Table 7. Number cases of isolation of all fungi in all 4 spices samples collected from Dakahlia centers, using seed health test.

Spices Fungi	Ginger	Nut meg	Paprika	Red chili	Total NCI for fungi	%Relative Density	%Frequancy of occurance	NSI and OR
Acremonium sp	3	0	7	0	10	14.08	10.98	10L
Alternaria sp	2	0	0	0	2	2.81	2.19	2R
Aspergillus	63	81	68	79	291	---	---	---
Aspergillus flavipes	0	14	5	0	19	26.76	20.87	19M
Aspergillus flavus	9	13	14	13	49	69.01	53.84	49H
Aspergillus fumgiatus	0	0	0	4	4	5.63	4.39	4R
Aspergillus glaucus	0	6	2	1	9	12.67	9.89	9L
Aspergillus niger	19	21	22	24	86	121.12	94.50	86H
Aspergillus nomius	9	5	13	7	34	47.88	37.36	34H
Aspergillus ochraceus	8	7	3	15	33	46.47	36.26	33H
Aspergillus oryzae	2	0	2	7	11	15.49	12.08	11L
Aspergillus parasiticus	8	5	7	3	23	32.39	25.27	23M
Aspergillus tamarii	7	1	0	5	13	18.30	14.28	13M
Aspergillus terrous	1	9	0	0	10	14.08	10.98	10L
Cephalosporum sp.	0	0	1	1	2	2.81	2.19	2R
Chaetomium sp.	0	0	3	4	7	9.85	7.69	7L
Emercella nomius	0	2	0	2	4	5.63	4.39	4R
Fusarium	5	10	20	12	47	---	---	---
Fusarium moniliforme	0	0	12	0	12	16.90	13.18	12M
Fusarium solani	4	10	5	10	29	40.84	31.86	29H
Fusarium oxysporum	1	0	3	2	6	8.45	6.59	6L
Penicillum sp.	20	18	9	8	55	77.46	60.43	55H
Phoma sp	0	0	2	1	3	4.22	3.29	3R
Rhizopus sp	6	5	18	16	45	63.38	49.45	45H
Trichoderma sp.	0	1	1	0	2	2.81	2.19	2R
Total NCI for spice	99	117	129	123	440	----	----	----
No. of genera	6	6	9	8	---	----	----	----
No. of species	14	14	18	17	----	----	----	----

NCI: Number of Cases of Isolation of fungi for each spice.

OR: occurrence remarks where H: high >24 , M: moderate 12-24, L: low 6-11, R: rare<6

The first important purpose of this investigation is the detection and determination of the synthesis of aflatoxins (namely B1, B2, G1, and G2) by strains belonging to the *Aspergillus flavus* group. A total of 71 isolates belonging to the *Aspergillus* flavus group were subjected to screening, and their concentrations were quantitatively determined using high performance liquid chromatography (HPLC) as illustrated in Figure(3). Moreover, the isolates were determined to possess toxigenic properties, with their capacity to manufacture aflatoxins ranging from 15 to 521 ng/ml of culture filtrate.

The first important purpose of this investigation is the detection and determination of the synthesis of aflatoxins (namely B1, B2, G1, and G2) by strains belonging to the *Aspergillus flavus* group. A total of 71 isolates belonging to the *Aspergillus* flavus group were subjected to screening, and their concentrations were quantitatively determined using high performance liquid chromatography (HPLC) as illustrated in Figure(3). Moreover, the isolates were determined to possess toxigenic properties, with their capacity to manufacture aflatoxins ranging from 15 to 521 ng/ml of culture filtrate.

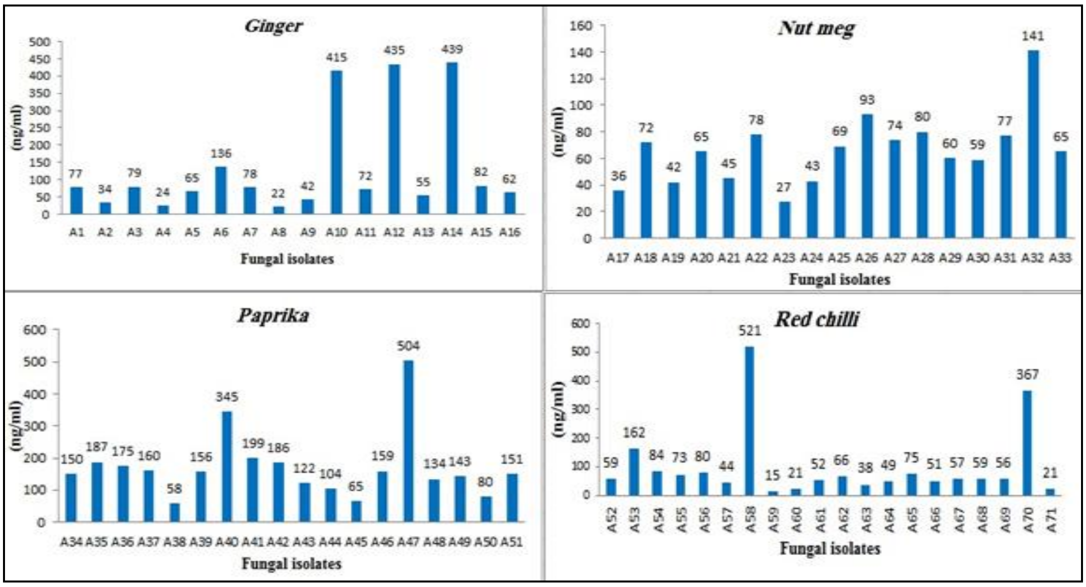

Figure 3. Aflatoxins production (ng/ml) of isolated *Aspergillus* sp in different studied samples.

Also, the highest value of total aflatoxin was 521ng/ml from *Aspergillus flavus* (No. 58) isolated from red chili samples. While, the lowest value was 15ng/ml from *Aspergillus oryzae* (No. 59) isolated also, from the investigated samples of red chili. Also, ginger samples examination demonstrated that, the 16 isolates were determined to possess toxigenic characteristics and had the ability to manufacture aflatoxins within a range of 22 to 439 ng/ml of culture filtrate. Furthermore, it was observed that the 17 isolates of nutmeg samples had the ability to generate aflatoxins within a range of 27 to 141 ng/ml of culture filtrate. The current Figure 3 indicated that, the 18 isolates obtained from the studied samples of paprika were detected to form aflatoxins with range of 58 to 504 ng/ml of culture supernatant. Furthermore, the 20 isolates gotten from the investigated samples of red chili were found to make aflatoxins in values ranging from 15 to 521 ng/ml of the filtrated culture.

A total of 71 isolates belonging to the *Aspergillus flavus* group were examined, revealing their capacity to manufacture aflatoxins within a range of 15 to 521 ng/ml of culture filtrate. In this regard, a study conducted by Azzoune *et al.* (2016) revealed that out of overall 151 isolated fungi related to the taxonomic group known as *Aspergillus flavus*, 67 were determined to be toxigenic. These isolates exhibited a wide range of aflatoxin production capability, with levels ranging from 0.1 to 818.2 ng/ml of culture supernatant.

Aspergillus flavus group isolates which from all studied spices were found to have toxigenic properties and had the ability to synthesize aflatoxins and the same results were in agreement with Fundikira *et al.* (2021) and Tosun and Arslan (2013) on Ginger; Akiyama *et al.* (2001) and Martins *et al.* (2001) on Nut meg; Erdogan (2004) and Kursun and Mutlu (2010) on Paprika and Hammami *et al.* (2014) & O'Riordan and Wilkinson (2008) on Red chili.

The utilization of multiple methodologies, such as morphological and molecular characterization, has gained increasing popularity and reliability in the identification and characterization of individuals belonging to section Flavi, passing the reliance on a singular approach Martins *et al.* (2001); Erdogan (2004). The utilization of ITS regions of nuclear ribosomal deoxyribonucleic acid has been comprehensively employed in the examination of the variability present within filamentous fungi, particularly to focus on the species and sub-species classifications Maina *et al.* (2019). The ITS region serves as a widely employed DNA barcode identifier for the purpose of distinguishing between various species in the field of molecular phylogenetic investigations Maina *et al.* (2019) and Henry *et al.* (2000) The presence of sequence diversity within the ITS sections has been demonstrated in both referenced and clinical isolates of *Aspergillus* species. The study conducted by Maina *et al.* (2019) determined that ITS-RFLP, which stands for restriction fragment length polymorphism, is a highly useful technique for the identification of nucleotide polymorphisms in *Aspergillus flavus* isolates. In addition, the utilization of nuclear ITS sequences has been suggested as a viable method for the detection of DNA barcode(s) of fungal species. Schoch *et al.* (2012)

In the case of ITS regions, the length of the sequence for each area is considered for the selected *Aspergillus flavus* isolate was 457 bp (MW246794) and the GC content for studied fungus was 58.2%. The BLAST search, available at https://blast.ncbi.nlm.nih.gov/Blast.cgi, yielded a pairwise identity (PI) value of 96.95% Table 8. The molecular phylogenetic tree result for the ITS 1 and the significance of the ITS2 regions in barcoding was demonstrated by their ability to capture phylogeographic differences. The evolutionary examination of the Internal Transcribed Spacer (ITS) gene is depicted in Figure 4.

Table 8. Accession number of the studied *Aspergillus flavus* produced Aflatoxin B1 isolated from studied spices in Egypt.

Fungi	Genbank Accession numbers	Pairwise Identity (PI)	Nucleotide (bp)					GC %
			A	T	G	C	Total	
Asperigillus flavus	MW246794	96.95%	91	100	129	137	457	58.2%

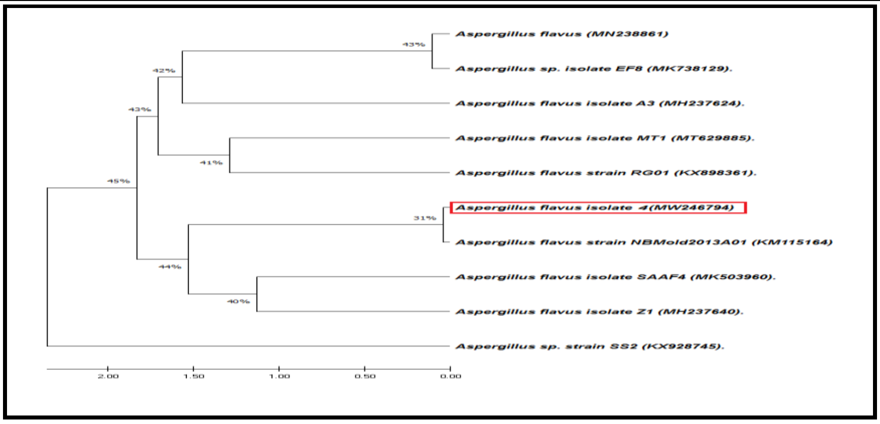

Figure 4. Phylogenetic tree for *Aspergillus flavus* isolated from spices in Egypt inferred from ITS sequences obtained from Gene Bank (highlighted in red color). Bootstrap tests were performed with 1000 replications.

The present work employed genotyping based on sequence changes in the ITS4-ITS5 region to validate the morphological identification of the chosen *Aspergillus* strain. Additionally, the utilization of nuclear ITS sequences has successfully validated the identification of the chosen *Aspergillus* strain. The obtained sequences was submitted to the National Center for Biotechnology Information (NCBI) GenBank and were assigned unique accession numbers as: MW246794.The aforementioned findings were also documented by (Henry et al. 2000), who employed sequence changes in the ITS1-5.8S-ITS2 region for the purpose of molecular identification of the five designated *Aspergillus flavus* strains, as well as to ascertain the genetic diversity present among these strains. The nuclear ITS sequences successfully facilitated the identification of the five *Aspergillus flavus* strains that were tested.

Various compounds, including essential oils and flavonoids, have the efficiency to prevent the synthesis and proliferation of *Aspergillus*, specifically in relation to aflatoxin. Alpsoy (2010). Essential oils are concentrated volatile liquids obtained from a variety of aromatic plants Masyita *et al.* (2022). Many biological properties, such as antifungal, antibacterial, anticancer, antioxidant, and anti-inflammatory activities, are exhibited for many Essential oils Minozzo *et al.* (2023). Essential Oils possess a high potential to inhibit microorganisms, particularly fungi, due to their bioactive compounds and biological functions. Therefore, they can be extensively employed as natural antimicrobial agents to preserve food quality and extend shelf life Abdi-Moghadam *et al.* (2023).These bioactive compounds which including terpenes and aroma compounds like phenols, hydrocarbons, aldehydes, alcohols, methoxy derivatives, and methylenedioxy compounds can exhibit potential biological activities, such as antibacterial and antifungal properties. The diverse phenolic groups within their structures make them suitable for use as functional, flavoring, and preservative agents in foods Dhifi *et al.* (2016). Abdel-Wahab (2007) studied the effect of 17 types of essential oils (clove, black seed, caraway, mint, thyme….etc.) in various concentrations as (0.01, 0.05, 0.1 and 0.5% v/v) were evaluate for their inhibitory activity on *Aspergillus flavus* and he found that most of the oils evaluated exhibited significant reduction effect on the growth of isolated fungi and production of Aflatoxin Abdel-Wahab (2007). In this respect, numerous publications have extensively established the antibacterial properties exhibited by essential oils and extracts of plant, such as rosemary, peppermint, basil, tea tree, and fennel. Hili *et al.* (1997)

Ten essential oils which were Green tea, Caraway, Cinnamon, Mint, Black seed, Basil, Sage, Rose mary, Clove and Thyme Table 9 and Figure 5&6 with different concentrations (0.05, 0.1, 0.2, 0.4 and 0.8% v/v) were tested for their capacity to suppress the growth of the selected Aflatoxin producing fungal isolate that was *Aspergillus flavus* after fixative incubation time as the dry weight were estimated at each concentration and inhibition ratio were calculated. Most of the oils evaluated exhibited significant reduction effect on the growth of fungus in overall concentrations with difference inhibition ratios.

The data revealed that, the growth of *Aspergillus flavus* was significantly decreased at all concentrations of the essential oil under investigation were in comparison with the group of control as inhibition ratio was 100% at 0.8% concentration at cinnamon, mint and clove essential oils. Besides, the results indicated that caraway had the least inhibition ration 9.4% , ranked in second saga and basil with the same reduction ratio 34.7%.

Data revealed that, *Aspergillus flavus* growth was has a significant reduction at all concentrations of investigated essential oil especially cinnamon, mint and clove essential oils. Khorasani *et al.* (2017) Revealed that extracts of cinnamon, clove, and celak shown promising capabilities in inhibiting the growth of *Aspergillus flavus*, affecting both its spores and mycelium. Furthermore, it was demonstrated by Abdi-Moghadam *et al.* (2023) that cinnamon oil, at doses of 2.0% (v/v) and 3.0% (v/v), exhibited effective inhibition of *Aspergillus flavus* growth.

Also, Thanaboripat *et al.* (1997) Detected the inhibiting impacts of garlic, clove, and carrot extracts on the development of *Aspergillus flavus*. The concentrations tested were 20,000, 40,000, 60,000, 80,000, and 100,000 µg/mL and its formation of aflatoxin. The findings of that study indicated that garlic, clove, and carrot possess inhibitory properties against *Aspergillus flavus* growth and the generation of aflatoxin. The concentration of garlic and clove at 100,000 µg/mL resulted in a significant decrease in aflatoxin levels, reducing them from 5.94 µg/g to 0.15 µg/g and 0.06 µg/g, gradually. Garlic, clove and carrot at 100,000µg/mL also inhibited the mould growth. Also, BOUDDINE *et al*. (2012) said that the antimicrobial activity of oregano and thyme oils against *Aspergillus* was shown to be quite strong and fungicidal agent. Moreover, Silva *et al.* (2012) studied the effect of essential oil mint on fungi as the efficacy of *Mentha piperita* L., a member of the Lamiaceae family, was assessed in relation to its inhibitory action againt the mycotoxin-producers fungi *Aspergillus flavus* and *Aspergillus parasiticus.*

Table 9. Effect of various concentration of studied Essential oils on the fungal growth of *Aspergillus flavus* isolate after fixative incubation time.

Treatment			Dry weight (Gm)	%Inhibition	Treatment			Dry weight (Gm)	%Inhibition
ID	Control		0.95 a	0.0	ID	Control		0.95 a	0.0
1	Green tea (v/v%)	0.05	0.89 ab	6.3	6	Basil (v/v%)	0.05	0.93 a	2.1
		0.1	0.78 c-e	17.8			0.1	0.81 b-e	14.7
		0.2	0.65 f-i	31.5			0.2	0.65 f-i	31.5
		0.4	0.61 g-j	35.7			0.4	0.59 h-k	37.8
		0.8	0.61 h-j	35.7			0.8	0.62 g-j	34.7
2	Caraway (v/v%)	0.05	0.88 ab	7.3	7	Sage (v/v%)	0.05	0.82 b-d	13.6
		0.1	0.72 e-g	24.2			0.1	0.76 de	20
		0.2	0.65 f-i	31.5			0.2	0.66 f-h	30.5
		0.4	0.65 f-i	31.5			0.4	0.60 h-j	36.8
		0.8	0.58 h-k	38.9			0.8	0.62 g-j	34.7
3	Cinnamon (v/v%)	0.05	0.72 d-f	24.2	8	Rose mary (v/v%)	0.05	0.89 ab	6.3
		0.1	0.66 f-h	30.5			0.1	0.76 de	20
		0.2	0.61 h-j	35.7			0.2	0.65 f-i	31.5
		0.4	0.63 f-j	33.6			0.4	0.62 f-j	34.7
		0.8	0 m	100			0.8	0.57 h-k	40
4	Mint (v/v%)	0.05	0.61 g-j	35.7	9	Clove (v/v%)	0.05	0.57 h-k	40.0
		0.1	0.59 h-k	37.8			0.1	0.60 h-k	36.8
		0.2	0.50 k	47.3			0.2	0.53 jk	44.2
		0.4	0.54 i-k	43.1			0.4	0.17 l	82.1
		0.8	0 m	100			0.8	0 m	100.0
5	Black seed (v/v%)	0.05	0.66 f-h	30.5	10	Thyme (v/v%)	0.05	0.86 a-c	9.4
		0.1	0.63 f-j	33.6			0.1	0.76 c-e	20.0
		0.2	0.63 f-j	33.6			0.2	0.63 f-j	33.6
		0.4	0.64 f-i	32.6			0.4	0.63 f-j	33.6
		0.8	0.60 h-k	36.8			0.8	0.61 h-j	35.7

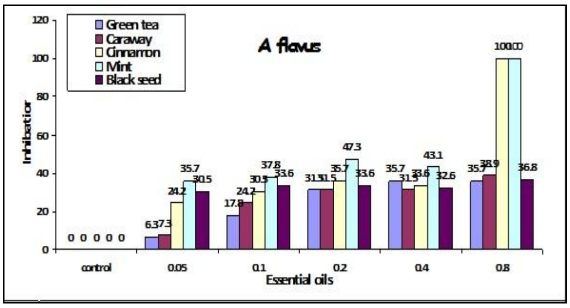

Figure 5. Effect of various concentration of Green tea, Caraway, Cinnamon, Mint and Black seed Essential oils on the *Aspergillus flavus* growth after fixative incubation time.

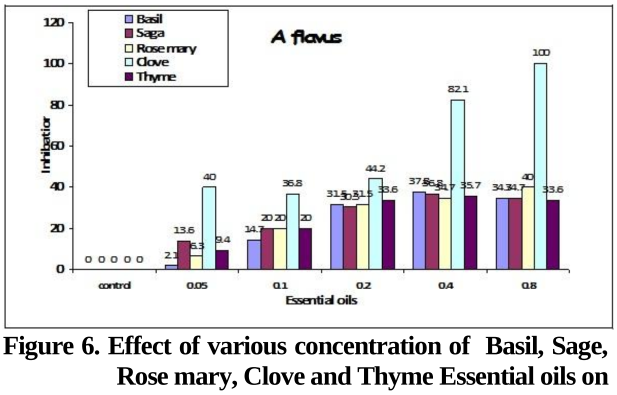

Figure 6. Effect of various concentration of Basil, Sage, Rose mary, Clove and Thyme Essential oils on the *Aspergillus flavus* growth after fixative incubation time.

Finally, Pinto *et al*. (2009) found that the application of clove oil and eugenol lead to a significant reduction in the concentration of ergosterol, a distinct component of the fungal cell membrane. The observed action of this oil can be attributed to its elevated doses (85.3%) of eugenol. Eugenol has been identified as a bioactive component of clove oil, with minimum inhibitory concentration recorded between 0.08 to 0.64 µl ml-1. Also, Sinha *et al.* (1993) studied the impact of varying amounts of clove and cinnamon oils on *Aspergillus flavus* growth and the formation of aflatoxin in SMKY liquid media. A statistically significant decrease ($P < 0.05$) in the production of aflatoxin in broth culture was seen following application of concentrations exceeding 100 µg ml-1 of these substances. The results of the study indicate that cinnamon oil exhibited a substantial inhibitory impact, with a maximum level of inhibition observed. Furthermore, the application of cinnamon oil resulted in a substantial reduction of 78% in aflatoxin generation.

CONCLUSION

Aflatoxins are classified as highly potent natural chemicals with carcinogenic, mutagenic, and teratogenic properties, commonly found in food and feed especially in spices. Essential oils are safe and inexpensive for biocontrolling *Aspergillus* species. The efficiency of ITS sequences for establishing the presence of genetic variation underscores the need for a deeper understanding of cluster construction.

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التواجد الطبيعى للفطريات المنتجة للأفلاتوكسين في بعض التوابل المصرية والمكافحة الحيوية بالزيوت الأساسية

بسمة فتحى محمد الجيوشى[١] ، محمد أحمد المتولى[٢] وماجدة إبراهيم سليمان[٣]

[١]مركز البحوث الطبيةالتجريبية (MERC)- كلية الطب- جامعة المنصورة.

[٢] قسم بحوث الفطريات - معهد بحوث أمراض النبات - مركز البحوث الزراعية - الجيزة.

[٣]قسم النبات - كلية العلوم- جامعة المنصورة.

الملخص

تم إجراء هذا المسح لتحديد وتعريف وعزل الفطريات بطريقة القياسية (SBM)، وخاصة الفطريات المنتجة للأفلاتوكسين؛ أنواع الرشاشيات*Aspergillus* المنتجة للأفلاتوكسين B1 وB2 وG1 وG2 وهي نواتج أيض ثانوية مسرطنة وسامة للإنسان والحيوان من إجمالي ٩١ عينة من البهارات المصرية (الزنجبيل وجوزة الطيب والفلفل الأحمر والفلفل الحلو) بأسواق الدقهلية و ١١ مستشفى ومركز طبي بالمنصورة. من بين جميع الأجناس المقدرة، كان لجنس الرشاشيات *Aspergillus* أعلى تواتر وانتشار، وهو ما يمثل ٢٩١ من إجمالي عدد الحالات التي تنطوي على عزل الفطريات. ثم جاء جنس *Pencillium* في المرتبة الثانية بنسبة ٥٥، ثم جنس *Fusarium* بنسبة ٤٧. كما تم فحص ٧١ عزلة من مجموعة *Aspergillus flavus*، وتم حساب تركيزات الأفلاتوكسين منها بإستخدام HPLC. علاوة على ذلك، تم تحديد أن العزلات تمتلك خصائص سامة، حيث تتراوح قدرتها على تصنيع الأفلاتوكسينات من ١٥ إلى ٥٢١ نانوجرام/مل من راشح المزرعة. تم تقديم التعريف الجزيئي عن طريق التشفير الشريطي ITS للحمض النووي للسلالة المدروسة إلى NCBI GenBank. كان طول التسلسل لفطر *Aspergillus* المحدد ٤٥٧ نقطة أساس، وكان محتوى GC 58.2٪. وكان التأثير التثبيطي باستخدام عشرة زيوت أساسية بتراكيز مختلفة كما يلي: الزيوت الأساسية الأكثر فعالية هي زيت القرنفل وزيت القرفة وزيت النعناع بنسبة تثبيط بنسبة ١٠٠٪ بتركيز ٠,٨٪ لجميعها.

الكلمات الدالة: الأفلاتوكسين , البهارات , الجزيئي و الزيوت الأساسية