Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR)
Faculty of Science, Assiut University, Assiut, Egypt.
Printed ISSN 2812-5029
Online ISSN 2812-5037
Vol. 54(1): 99- 118 (2025)
https://aunj.journals.ekb.eg

Control of mycobiota and mycotoxins contaminating popcorn grains

Mohamed Hamdy,[2*] Ahmed M. Moharram[1,2] and Abdel-Naser A. Zohri[1]

1- Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71511, Egypt
2- Assiut University Moubasher Mycological Centre (AUMMC), Assiut University, Assiut, Egypt
*Corresponding Author: mhy1002004@yahoo.com

ARTICLE INFO

Article History:
Received: 2024-09-09
Accepted: 2024-11-12
Online: 2024-12-26

Keywords:
Popcorn Grains, Toxigenic Fungi, Mycotoxins, control agents, Spices, Essential Oils

ABSTRACT

Sixty-six species and one varity affiliated with 22 genera were isolated from 20 samples of popcorn grains collected from different stores in Assuit Governorate during four seasons (winter 2018 to Autumn 2019) on dichloran rose bengal chloramphenicol agar (DRBC) and dichloran glycerol agar (DG18) media. The most common genera were *Aspergillus* followed by *Fusarium*, *Cladosporium* and *Penicillium*. The most abundant species were *Aspergillus niger*, *A. flavus*, *Cladosporium cladosporioides* and *Fusarium oxysporum*. All collected samples of popcorn were free from detectable amounts of mycotoxins. Among 30 isolates represented 3 species (*Aspergillus flavus*, *A. versicolor* and *Fusarium verticilloides*): only two isolates of *Aspergillus flavus* were potent to produce Aflatoxin B_1. The inhibitory action of spices and essential oils against the growth of isolated aflatoxin producers and their abilities to produce aflatoxin as control agents, were examined. Clove was found to be the most active spice which entire prevented the growth of the tested fungal isolates at concentration of 1.0 %. On the other hand, neither of the two fungal strains produced any detectable amount of aflatoxin in the presence of either of the tested or the two essential oils. These results could be advantageous for rustic folk to block the contamination of fungi and their toxins in different grains and/or seeds by a simple, safe and low-cost method.

INTRODUCTION

Popcorn (*Zea mays* L. *everta*) in India, Central and North America, is the predominant variety of maize [1]. The spread and diversification of maize around the world led to an increase in popcorn production, consumption, and awareness. Popcorn is imported from odd countries into Egypt due to the country's increasing demand for it. Consequently,

popcorn costs four times as much as regular corn grain in Egypt [2]. United States produced more than 400 million kilograms of popcorn in the year 2005 [3].

Popcorn is subject to contamination by mycotoxins [4]. Because popcorn is consumed by humans, tolerance levels for mycotoxins are typically lower in popcorn than for dent maize that is used for animal feed [5].

The rapid increase in the global population and the expansion of the primary crop planting area has resulted in an annual rise in mycotoxin contamination in agricultural goods [6]. Foods have been preserved using a variety of chemical and physical techniques, but because of their drawbacks, these techniques are banned in many modern nations [7]. As a result, natural and safer food preservatives have just lately become popular worldwide [8]. Since ancient times, people have added spices to meals as flavoring agents, traditional remedies, and food preservatives [9, 10]. In addition to its anticancer properties, it is utilized as a remedy for minor medical issues such colds, motion sickness, stomach problems, ulcers, and muscle aches [11].

Certain spices not only contribute distinctive scents to food, but they also extend its shelf life by preventing rancidity via their antioxidant or antibacterial abilities [12]. Due to extensive toxicological research or their historical use, spices are generally regarded as harmless [13].

Since spices are natural components, they are resumption to many customers who ask about the safety of artificial food additives. Some of the spices that are used today are prized not just for their great flavor and scent but also for their antibacterial properties and medicinal effects. In recent years, there has been a surge interest in spice extracts due to its bioactive properties, which can be used in a variety of pharmaceutical and food processing applications. These days, the benefit in recognizing naturally occurring substances, like spices, can prevent or inhibit the growth of toxic fungi and/or their capacity to create mycotoxin [14, 15]

Essential oils (EOs) are plant-based essential extracts that possess potent antibacterial, antitoxigenic, and food preservation properties, along with low toxicity to humans and animals [16]. Clove has been used as a spice in cooking as well as a medical tool in the field of herbal medicine. The flavoring agent and source of the plant's essential oil are the flower buds. Its essential oil is unique in that it has potent aromatic scents and is highly volatile. Thus, aromatherapy and cosmetics have been strongly associated with clove essential oil. In dentistry, it has also been utilized as a natural drugged [17].

Clove essential oil was found to be potent against ***Aspergillus parasiticus***, ***Candida albicans,*** and ***Cryptococcus neoformans***, [18]. Therefore, the purpose of this study was to identify the mycobiota and mycotoxin that contaminated popcorn made from grains of maize. Determine if high-activity spices and essential oils may stop the growth of certain toxigenic fungus isolates and their formation of mycotoxin.

MATERIALS AND METHODS

- **Collection of Samples**

Twenty samples of popcorn grain were purchased from local supermarkets and grocery stores in Assuit, Egypt during the course of four seasons, spanning from winter 2018 to autumn 2019 (five samples each season, one kg of each sample). After being collected, they were kept in their original package at room temperature and examined within a week.

- **Determination of moisture content**

By using the techniques outlined in [19], the moisture content was determined by weighing samples of 10 grams which kept at 105 ^{O}C in an electric oven until constant weight.

- **Isolation of fungi:**

Dichloran rose bengal chloramphenicol agar medium (DRBC) was utilized for fungal isolation [20]. IT is composed of (g/l) peptone 5.0, potassium dihydrogen phosphate 1.0, Mg SO_4 0.5, glucose 10.0, and agar 15.0. Dichloran (20 µg/ml) and rose bengal (25 µg/ml) were added as bacteriostatic agents, along with chloramphenicol (100 µg/ml). Dichloran 18% glycerol agar (DG18) was used as a selective medium for isolating xerophilic fungi. It contains the following ingredients in grams per liter: peptone 5, glycerol 220, chloramphenicol 0.1, glucose 10, potassium dihydrogen phosphate 1, Mg SO_4 0.5, dichloran 0.002 (0.2% in ethanol 1 ml), agar 15, and final pH 5.6. A description of DG18 and DRBC media was provided by [23].

Two techniques were used to count the fungal flora in the samples: direct plating method [24] as follows: ten grams of popcorn were surface-sterilized for one minute in 5% NaOCl, and then the samples were twice rinsed in sterile distilled water. The grains were aseptically placed onto the solidified agars after being surface sterilized. Each sample was plated on a total of 5 plates. Each agar plate had five grains put on it. The Dilution Procedure Approach [25] was employed to count and isolate fungus from popcorn kernels. In 250 ml Erlenmeyer flasks, 10 g of popcorn from each sample was suspended in 90 ml of sterile water. For thirty minutes, the flasks were mechanically shaken. Five sterile petri dishes were filled with one milliliter of the dilution, and each was then filled with roughly twenty milliliters of cooled agar medium that had been brought just above the temperature of consolidation. The inoculated plates were incubated at 28 oC for seven days, during which the colonies were counted. For identification, the various fungal colonies on the plates were subculture on potato sucrose agar (PSA) medium

- **Identification of filamentous fungi**

The relevant and reliable references [26-37] were used for the identification of the collected fungal genera and species.

- **Mycotoxins extraction**

A blender jar was filled with 50 grams of blended popcorn from each of the 20 samples that were collected. 100 milliliters of chloroform was then added, and the

contents were homogenized for 5 minutes at low speed and 3 minutes at high speed. Whatman No. 1 filter paper was used to filter the extract. The same amount of chloroform was used twice for the extraction process. The mixed chloroform extracts were dried with anhydrous sodium sulphate, rinsed with an equal amount of distilled water, and then evaporated on a steam bath until almost dry. One milliliter of chloroform was added to a tiny vial containing the residue [38].

According to Aziz *et al.* [39], the ability of the chosen fungal isolates to generate toxic metabolites in liquid cultures on potato sucrose medium was evaluated. The medium included 500 ml of potato extract, 20 g of sucrose, 20 g of agar, and 500 ml of distilled water (g/l). Next, autoclaving was carried out for 15 minutes at 121 ^{O}C. After 50 milliliters of media were added to 250 milliliter flasks, the flasks were inoculated with approximately 10^6 spores per milliliter of fungal isolate and shaken (200 rpm) for ten days at 28 ^{O}C. After incubation, the fungal cultures were mixed with 120 ml of chloroform: water (100: 10, v/v) and they were violently shaken all night long at 200 rpm using a rotary shaker. Anhydrous sodium sulfate was used in a progressive filtration process for the extracts. After gathering the chloroform extracts, they were almost completely dry on a steam bath, dissolved in one milliliter of chloroform, and then frozen until analysis.

- **Detection of mycotoxins**

Mycotoxins detection were carried out using thin layer chromatography (TLC) [40]. In a UV chamber, 50 microliters of the mycotoxin's chloroform extract were applied to silica gel plates together with particular standards made using mobile phase chloroform methanol (96: 4). The samples were then exposed to long-wavelength UV light (365 nm). Using the values of their retention factor and fluorescence, mycotoxins were qualitatively detected [41].

- **Effect of spices on growth of toxigenic fungal isolates**

Two varieties of spices (cumin and clove) were bought and dehydrated for four days at 65 ^{O}C in a hot oven. Following the procedure outlined by [15], the dried spices were ground to almost powder and their effects on the growth of two aflatoxin-producing fungus strains (AUMC 16585 & 16586) were investigated. The dried spices were employed in three different concentrations (1, 5 and 7 g / L medium). Before the spices solidified, they were put to sterile PDA flasks. On potato sucrose agar (PSA) medium, fungi were cultivated in Petri plates using one of the three spice concentrations that were examined. At 28 ^{O}C, petri plates were incubated. After five days of incubation, the diameter of the fungal colonies was measured to determine the impact of the spices, and this measurement was compared to the control culture, which was made of untreated PDA media.

- **The effect of essential oils on growth of toxigenic fungal isolates**

According to Ficker *et al.* [42], a disc diffusion test was used to determine the essential oils' antifungal effects (thyme and clove oils). One hundred µL of the isolated fungal culture with sterilized distilled water were transferred and spread onto potato - sucrose agar plate before being left to dry. A sterile filter paper disc, measuring approximately 11 mm in diameter, was pipetted with twenty microliters of each of the three concentrations of the two essential oils (0.1, 0.5, and 1.0 %) and positioned at the center of the plate. At 28 ^{O}C, plates were incubated. After five days of incubation, the diameter of the inhibitory zones was measured to determine the impact of the essential oils.

- **The effect of spices and essential oils on aflatoxins production**

In this experiment, two spices—cumin and clove—as well as two essential oils—thyme and clove—that have been shown to have an impact on fungal development were used. A potato-sucrose liquid medium supplemented with 0.5% of either of the two spices or essential oils was used to culture the fungal isolates. The medium in 50 ml aliquots was moved to 250 ml conical flasks. After 20 minutes of sterilization at 121 ^{O}C, the flasks were cooled and then injected with two milliliters of an inoculum suspension from the investigated fungal isolated.

The cultures were incubated for 10 days at 28 ºC. Following the incubation time, 100 ml of chloroform was added to the culture broth medium and homogenized for five minutes in a high-speed (16000 rpm) blender. The chloroform extracts were separated and then dried over anhydrous sodium sulphate, filtered, and concentrated until almost dry. They were then rinsed with an equivalent volume of distilled water. Mycotoxin levels were detected using thin layer chromatography [40, 41, & 43].

RESULTS

- **Moisture content**

Moisture content **(**M.C.) of the 20 popcorn ground samples ranged from 8.4 to 12.8 %. This result was nearly matches with the finding of [44]. They recorded that the moisture content of 30 samples of popcorn was ranging from 12.7 - 14.2 %.

- **Mycobiota**

Using the dilution plate technique and direct plate method at 28°C, on Dichloran Glycerol agar (DG18) and Dichloran Rose Bengal Chloramphenicol agar medium (DRBC), 66 species and 1 variety of filamentous fungi were recovered, they related to 22 genera. The total number of propagules fungi isolated on Dichloran Glycerol agar using dilution method (1331 CFUs / mg dry grains in all samples) was more than those obtained on DRBC (517 CFUS) using the same isolation method. On the other hand, on

DRBC and DG18 using direct plating method, the counts were 358 and 528 CFUs/ 100 grains, respectively. The species diversity on DRBC using dilution method was also higher (12 genera, 38 species) than those recorded on DG18 (11 genera, 35 species) by the same dilution method. The most prevalent fungal genera were *Aspergillus, Fusarium, Cladosporium, Penicillium, Alternaria* and *Trichoderm*a (Tables 1 and 2).

Using MEA, DRBC, and DG18 medium, similar results were obtained [44] *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium* were the most common genera of mycobiota in 30 samples of maize flours and 30 samples of popcorn seeds purchased from Spain for human consumption.

Additionally, [45] discovered that *Aspergillus*, *Fusarium*, and *Penicillium* were the three most prevalent fungal genera in samples of recently harvested and preserved maize seeds.

Mahmoud *et al.* [46] examined 48 samples of maize and corn-derived products in Egypt to detect the presence of fungi. They identified 37 fungal species and one variety that were associated with nine different fungal genera. They discovered that while *A. niger* and *A. terreus* were noted for their moderate occurrence on popcorn and cornflakes, *Aspergillus flavus* and *A. paraciticus* were often the most common species in entire samples.

The results presented in Tables (1 & 2) clearly showed that ***Aspergillus*** (20 species + 1 variety), was recorded in 18 and 19 samples in DRBC and DG18 using dilution method and 18 and 17 by direct plating method out of 20 samples contributing 55.13%, 67.7% and 19.27 and 35.42 of total fungi on DRBC, DG18 using dilution media and DRBC, DG18 by direct platting method, respectively. Whereas, *A. flavus* was isolated in high frequency on only DG18 medium using dilution method and frequency in moderate on the other medium and method of isolation. *Aspergillus amstelodami, A. chevalieri* and *A. rubrum* were identified in moderate frequency on DG18 medium by the two methods of isolation but *A. montevidensis* and *A. candidus* were isolated in moderate frequency in DG18 by dilution method only. *Aspergillus ochraceus* was recovered in low frequency in DRBC and with low frequency in DG18 using dilution method and with rare frequency in the two media by direct plate method. *Aspergillus versicolor* was recorded in low frequency in the two media by using dilution method and in rare frequency in the two media in the other method. *A. terreus* was isolated in low frequency in DG18 in dilution method only. *Aspergillus ustus* was recorded with low frequency in DG18 by dilution method. The remaining *Aspergillus* species (*A. awamori, A. clavatus, A. fumigatus, A. fumigatus* var. *albus, A. nidulans, A. tamarii, A. oryzae, A. parasiticus, A. rugulosa* and *A. wentii)* were recovered in rare frequency of occurrence. As general, *A. niger* and *A. flavus* were the most common species in popcorn grains in Assuit (Tables, 1&2).

Seven genera were recovered from corn samples by Roige *et al.* [47] in southeastern Buenos Aires province, Argentina, *Aspergillus* was presented in 34% CFUs of all

recovered genera. In their study on the occurrence of mycobiota in samples of freshly harvested and stored maize kernel [45] could identified *A. flavus* with higher incidence in the stored kernels than in freshly harvested kernels. Whereas *A. parasiticus* was the predominant species in the freshly harvested kernels compared to the stored kernels. *Aspergillus* is a common genus ranged from 29.9 - 61.3 % and 55.9 – 84.1 % of total fungal counts in Giza and Behira (Egypt) samples, respectively [48]. *Aspergillus flavus* and *A. parasiticus* were common species from 48 different corn and corn-based products samples in Egypt [46].

Fusarium (8 species) was the second common genus. It was registered in high frequency in the both employed media using the two isolation methods. It was recorded in 14 and 11 samples contributing 29 and 22% of the total fungal counts in DRBC and DG18 by dilution method and in 17 out of 20 samples (52.5% and 18.94%) from DRBC and DG18 by direct plate method respectively. *Fusarium oxysporum* was recorded in high frequency in DRBC by direct plate technique only but it was moderate frequency using the other medium and method. *Fusarium solani* and *F. verticillioides* were isolated in moderate frequency regardless to the employed culture media and the isolation method. *Fusarium semitectum* and *F. subglutinans* were isolated in low frequency in DG18 by direct plate method only but were in rare frequency in the others. The other 3 species of *Fusarium* (*F. equiseti, F. pseudoanthophilum* and *F. sporotrichioides* were recovered in rare frequency. (Table 1&2).

F. oxysporum was the most abundant *Fusarium* species appeared in 28% of the samples constituting 1.6% of total fungi in maize grains, in Assiut, Egypt [49]. Seven genera from corn samples in Southeastern Buenos Aires Province, Argentina was recovered by Roige *et al.* [47] and found that the *Fusarium* was the most common of all genera recovered.

According to Adejumo *et al.* [50], who studied 180 maize samples meant for human consumption from four states that produce maize in southwest Nigeria, found that *F. verticillioides* was the most frequently isolated fungus, followed by *F. sporotrichioides, F. graminearum, F. pallidoroseum, F. compactum, F. equiseti, F. acuminatum, F. subglutinans,* and *F. oxysporum*.

In a research on the presence of mycobiota in fresh and preserved maize kernels in Belgrade, Krnjaja *et al.* [45] identified three species of *Fusarium*: *F. graminearum, F. subglutinans, and F. verticillioides.* Mahmoud *et al.* [46] found low or infrequent occurrences of *F. avenaceum, F. oxysporum, F. phyllophilum, F. poae, F. proliferatum, F. pseudonygamai, F. solani, F. sterililyphosum, and F. verticillioides* in their investigation on corn and corn-based product samples in Egypt.

Cladosporium (*C. cladosporioides* and *C. sphearospermum*) was the third genus recovered from popcorn. It was recovered in high frequency of occurrence using the two media and isolation methods except in DRBC by dilution method; it was recorded in

moderate frequency. *Cladosporium* was recorded in 7 & 13 samples by dilution method and 14 & 18 out of the 20 tested samples by direct plate method in DRBC & DG18, yielding 2.5, 5.89, 18.7 and 36.9 % of total fungal count, respectively. *Cladosporium cladosporioides* was the most prevalent species. It was isolated in moderate frequency in DRBC using dilution method and in high frequency using the other medium and the other method of isolation. *Cladosporium* presented in 9% CFUs of all seven fungal genera recovered from corn samples in Southeastern Buenos Aires Province, Argentina. [47]. *Cladosporium* spp. found in 20% of 30 samples of popcorn seeds bought in Spain for human uses [44].

Penicillium (9 species) was recovered in a moderate frequency using the both media and the two isolation methods. It was recorded in 10, 8, 7 and 6 out of 20 samples yielding 7.74, 3.01, 3.07 and 1.52% of total fungal count in DRBC and DG18 using direct plating and dilution method, respectively. The most frequent species was *P. duclauxii* (moderate frequency of occurrence in DRBC but with low frequency in DG18 using dilution method). It was low and rare frequency of occurrence using DRBC, and DG18 by the direct plating method respectively. *P. chrysogenum* was occurred with moderate frequency in DG18 by dilution method but with rare occurrence using the other medium. *P. oxalicum* was isolated in low frequency in DG18 by dilution method but with rare occurrence frequency in the other. The remaining species (*P. digitatum, P. expansum, P. funiculosum, P. variabile, P. verrucosum* and *P. purprogenum*) were registered in rare frequency in DRBC and DG18 by using the two different method of isolation.
In their study on the occurrence of mycobiota in freshly and stored maize kernels [45] found that the species of *Penicillium* had higher incidence in freshly kernels than in the stored kernels. Seven genera from corn samples in Southeastern Buenos Aires Province, Argentina and found that *Penicillium* was presented in 70% CFUs of all genera recovered [47].

Alternaria (*A. alternata* and *A. chlamydospora*) was isolated in moderate frequency using the direct plate method on the both employed media but was isolated with rare frequency using the other isolation method. It was present in 1 and 2 out of 20 samples using dilution method on DRBC and DG18, respectively. *Alternaria* spp. was isolated in rare frequency out of 30 popcorn seeds bought in Spain for human uses [44].

Stemphylium botryosum was recorded with low frequency in DG18 and with rare on DRBC using dilution method and with rare on DRBC and disappear on DG18 using direct plate method. *Trichoderma harzianum* was recorded with low frequency on DRBC using dilution plate technique only and disappear in the other medium and isolation method. The remaining genera and species listed in the Tables 1 and 2 were isolated in rare frequency of occurrence.

- **Natural occurrence of mycotoxins**

All twenty samples of popcorn were analyzed for natural occurrence of mycotoxins and thin layer chromatography detection appeared that all the tested samples were free from any detectable amounts of mycotoxins. This may be due to the popcorn was new or current season products or stored in a good condition. Present data and that of Kos *et al.* [51] findings are quite similar, they examined 130 grain samples from Serbia for aflatoxins, they discovered that only 24 out of the 40 tested samples of maize were contaminated. In contrast, none of the tested samples of wheat, barley, oats, or rye had aflatoxins present. Furthermore, the natural co-occurrence of aflatoxins in Egyptian corn samples was documented by Deabes *et al.* [52], according to their findings, nine of the thirty corn grain samples that were deemed reliable to be from the Cairo governorate (B1, B2, G1, and G2) were infected with AFs. Whereas AFs were found in 7 out of 30 corn samples that were taken from the Giza Governorate.

- **Mycotoxins produced by tested fungal isolates**

Thirty common fungi were isolated in this investigation, including twelve strains of *Aspergillus flavus*, ten strains of *A. versicolor*, and eight strains of *Fusarium verticillioides*, were evaluated for their ability to produce mycotoxin in a synthetic medium. However, aflatoxin B1 produced by two isolates of *A. flavus* (AUMC 16585 and 16586) to varying degrees. The formation of mycotoxins was detected using the TLC technique, and the results showed that none of the isolates of *A. versicolor* and *F. verticillioides* that were investigated were able to produce measurable amounts of mycotoxins under experimental conditions. Alborch *et al.* [44] reported that 13 of the 42 strains of *A. flavus* strains were found in maize might create aflatoxins in this regard.
Aflatoxins, ochratoxin-A, fumagillin, and terrein were discovered to be produced by twenty-seven fungal isolates with affinity for *A. flavus* (21 isolates), *A. ochraceus* (3), *A. fumigatus* (1), and *A. terreus* (2) identified from rice in Egypt [53]. Aflatoxin production varied throughout *A. flavus* isolates.

- **Control agents**

The two aflatoxigenic fungal isolates (A. flavus AUMC 16585 & A. flavus AUMC 16586) were evaluated for their ability to grow and produce aflatoxin. The biocontrol agents, clove and cumin, were found to have this effect (Table 3). At a concentration of 0.5% (w/v), clove was shown to be the most effective spice, and inhibited the development of while 1.0% clove spice entirely inhibited the growth of the *A. flavus* (AUMC 16586) (Table 3). Conversely, neither of the two fungal strains able to produce any measurable level of aflatoxin when grown in the presence of the studied spices. This outcome is almost identical to the results reported by [15 and 54].

Clove was the most effective against three isolates of toxigenic fungus, *A. flavus*, *A. versicolor*, and *P. citrinum*, which produced aflatoxins, sterigmatocystin, and citrinin, respectively, among the ten types of spices studied in Saudi Arabia by Bokhari [54]. Additionally, Zohri *et al.* [15] investigated the potential of nine Egyptian spices to inhibit the growth and production of toxins by certain toxic fungi. They found that clove was the most effective spice, completely blocking the fungi's ability to growth and produce mycotoxins at concentrations of 0.5 and 1% (w/v).

In this regard, numerous researchers around the globe have confirmed the antibacterial properties of various spices [55–60].

Awuah and Ellis [61] examined the efficacious use of clover leaf powder in combination with certain wrapping materials to preserve groundnut kernels in Ghana that had been intentionally contaminated with *A. parasiticus*. [55–60].

Juglal *et al.* [62] discovered that clove significantly reduces the production of aflatoxin in infectious grains in South Africa. These findings may help rural people take easy precautions against the synthesis of fungal toxins in contaminated grains [60].

A disc diffusion method was used to assess the two essential oils (thyme and clove) as biocontrol agents against the growth of the two aflatoxigenic fungus isolates and their capacity to manufacture aflatoxin (Table 4). The biggest inhibitory zone (mm) and a robust antifungal impact against the two tested fungi were demonstrated by clove oil at a concentration of 1.0%, although a partial effect was observed against the tested fungi at a concentration of 0.5%. However, in the presence of each of the two essential oils under investigation, the production of aflatoxin by each of the two fungal isolates was totally suppressed.

In this regard, Mostafa [63] investigated the antifungal activity of clove oil on *Candida albicans*, *Aspergillus flavus*, *Trichophyton mentagrophytes*, and *Microsporum canis* in Egypt. Their results showed that the clove oil exhibited potent antifungal activity against all of the tested fungi, particularly dermatophytes spp.

The effect of clove and cumin spices as well as the essential oils of clove and thyme on aflatoxin production by the two fungal isolates examined was carried out and the results clearly appeared that the concentrations of each of the two spices or the two essential oils under tests were completely inhibited aflatoxins formation. Nearly similar results were recorded by several researchers all over the world. On this respect, in Saudi Arabia Bokhari [54] reported that five spices (black pepper, peppermint, cardamom, cumin and marjoram) were completely inhibited both aflatoxin and sterigmatocystin production.

Additionally, Bokhari and Aly [60] found that either cloves or cinnamon can inhibit growth of *A. flavus* (by 50%) however aflatoxin production (by 100%).

At 0.8 mg/ml, caraway demonstrates antifungal efficacy against *A. parasiticus* and totally prevents them from producing aflatoxin [64].

At lower dosages, cumin was fungistatic, but at larger doses, it was fungicidal against strains of *A. flavus* and *A. parasiticus* that produce aflatoxin [65].

Twenty four commercial spices were tested for their ability to inhibit the production of toxins by two toxigenic *Aspergillus* species (*A. flavus* and *A. versicolor*) and two *Penicillium* species (*P. citrinum and P. corylophilum*). The results showed that thyme, Chinese cassia, cinnamon, clove, and chrysanthemum completely prevented the growth of the tested fungal isolates [66].

Banso *et al.* [67] in Nigeria found that Clove extract inhibited fungal proliferation at higher concentrations, reducing mycotoxin generation. As clove extract concentration grew from 10 mg/ml to 40 mg/ml, *A. nidulans* mycelia multiplication decreased from 8% to 98%. As extract concentration increased from 10 mg/ml to 50 mg/ml, *F. oxysporum* decreased ranged from 53% to 100%.

Zohri *et al.* [15] found that clove was the most effective spice, completely blocking the growth and mycotoxin production at a concentration of 0.5 (w/v) and completely inhibiting fungal count and mycotoxin production when used as a food additive at a concentration of 1% (w/v). The study examined the inhibitory effects of nine spices against some toxigenic fungi and their abilities to produce toxins. Additionally, they discovered that, at concentrations of 1%, black pepper, cinnamon, spearmint, cumin, thyme, and anise all totally blocked the synthesis of mycotoxins and prevented the growth of the majority of fungal isolates.

Table (1): Colonies forming units (CFUs / g) and frequency of occurrence (FO) of fungal genera and species recovered from Popcorn seeds on DRBC and DG 18 using dilution plate method at 28 °C.

Media / Fungal taxa	DRBC				DG 18			
	CFUs	% CFUs	FO	OR	CFUs	% CFUs	FO	OR
Acremonium strictum	3	0.58	1	5				
Alternaria alternata	1	0.19	1	5	2	0.15	2	10
Aspergillus	285	55.13	18	90	901	67.7	19	95
A. amstelodami					122	9.17	8	40
A. awamori					1	0.08	1	5
A. candidus	3	0.58	1	5	15	1.13	5	25
A. chevalieri	78	15.09	2	10	345	25.92	6	30
A. clavatus	1	0.19	1	5	1	0.08	1	5
A. flavus	63	12.19	10	50	94	7.06	11	55
A. fumigatus	6	1.16	2	10	1	0.08	1	5
A. ochraceus	6	1.16	3	15	2	0.15	2	10
A. oryzae	1	0.19	1	5	1	0.08	1	5
A. montevidense	18	3.48	1	5	23	1.73	5	25
A. niger	98	18.96	14	70	152	11.42	12	60
A. parasiticus	4	0.77	1	5				

Table (1): Continued

Media / Fungal taxa	DRBC				DG 18			
	CFUs	% CFUs	FO	OR	CFUs	% CFUs	FO	OR
A. rubrum	1	0.19	1	5	118	8.87	7	35
A. terreus					16	1.20	3	15
A. ustus	1	0.19	1	5	4	0.30	4	20
A. versicolor	4	0.77	3	15	6	0.45	4	20
A. wentii	1	0.19	1	5				
Aureobasidium pullulans	1	0.19	1	5				
Chaetomium spiralis	1	0.19	1	5				
Cladosporium	13	5.87	7	35	78	5.9	13	65
C. cladosporioides	12	2.32	7	35	71	5.33	12	60
C. sphearospermum	1	0.19	1	5	7	0.53	3	15
Curvularia lunata					1	0.08	1	5
Fusarium	150	29.01	14	70	294	22.09	11	55
F. equiseti	2	0.39	2	10				
F. oxysporum	27	5.22	6	30	193	14.50	8	40
F. pseudoanthophilum					1	0.08	1	5
F. semitectum	4	0.77	1	5				
F. solani	15	2.90	5	25	51	3.83	6	30
F. sporotrichioides	2	0.39	1	5	2	0.15	1	5
F. subglutinans					2	0.15	1	5
F. verticillioides	100	19.34	5	25	45	3.38	8	40
Mortierella alpine					3	0.23	2	10
Myrothecium verrucaria					1	0.08	1	5
Penicillium	40	7.74	10	50	40	3.01	8	40
P. chrysogenum	6	1.16	2	10	20	1.50	5	25
P. digitatum	2	0.39	2	10	1	0.08	1	5
P. ducluxii	24	4.64	5	25	11	0.83	4	20
P. expansum	1	0.19	1	5				
P. funiculosum	1	0.19	1	5				
P. purprogenum					3	0.23	2	10
P. oxalicum	4	0.77	2	10	5	0.38	3	15
P. variabile	1	0.19	1	5				
P. verrucosum	1	0.19	1	5				
Rhizopus oryzae					3	0.23	1	5
Scopulariopsis chartarum					1	0.08	1	5
Setosphaeria rostrata	1	0.19	1	5				
Stemphyllium botryosum	1	0.19	1	5	7	0.53	3	15
Trichoderma harzianum	19	3.68	4	20				

Table (1): Continued

	DRBC				DG18			
	CFUs	% CFUs	FO	O%	CFUs	% CFUs	FO	O%
Yeasts	2	0.39	2	10				
Total count	517	100	20	100	1331	100	20	100

CFUs= Colony forming units /100 plates in all seasons, % CFUs= calculated to total counts, FO= Frequency of occurrence, O%= Occurrence % = (No. of isolates / Total no. of isolates) * 100.

Table (2): Colonies forming units (CFUs / 100 seeds) and frequency of occurrence (FO) of fungal genera and species recovered from Popcorn seeds on DRBC and DG 18 using direct plating method at 28 °C.

Media / Fungal taxa	DRBC				DG18			
	CFUs	% CFUs	FO	O%	CFUs	% CFUs	FO	O%
Acremonium strictum	4	1.12	1	5	8	1.52	2	10
Alternaria	8	2.23	5	25	15	2.84	8	40
A. A. alternata	8	2.23	5	25	14	2.65	8	40
A. chlamydospora					1	0.19	1	5
Aspergillus	69	19.27	18	90	187	35.42	17	85
A. amstelodami					11	2.08	5	25
A. candidus					1	0.19	1	5
A. chevalieri	5	1.40	2	10	89	16.86	9	45
A. flavus	9	2.51	7	35	12	2.27	7	35
A. fumigatus	3	0.84	2	10	1	0.19	1	5
A. fumigatus var. Albus					1	0.19	1	5
A. montevidense					3	0.57	3	15
A. ochraceus	1	0.28	1	5	1	0.19	1	5
A. oryzae	1	0.28	1	5				
A. nidulans	1	0.28	1	5				
A. niger	45	12.57	11	55	50	9.47	9	45
A. rubrum					11	2.08	5	25
A. rugulosa	1	0.28	1	5				
A. tamarii					4	0.76	2	10
A. terreus	2	0.56	1	5	2	0.38	2	10
A. ustus	1	0.28	1	5	1	0.19	1	5
Botryotrichum piluliferum	1	0.28	1	5	1	0.19	1	5
Cladosporium	67	18.72	14	70	195	36.93	18	90
C. cladosporioides	65	18.16	13	65	175	33.14	16	80
C. sphearospermum	2	0.56	1	5	20	3.79	8	40
Chaetomium globosum	1	0.28	1	5				
Cochliobolus	1	0.28	1	5	R	0.19	1	5
C. sativus					1	0.19	1	5

Table (2): Continued

Media / Fungal taxa	DRBC				DG18			
	CFUs	% CFUs	FO	O%	CFUs	% CFUs	FO	O%
C. spicifer	1	0.28	1	5				
Curvularia australiensis	1	0.28	1	5				
Fusarium	188	52.51	17	85	100	18.94	17	85
F. oxysporum	134	37.43	14	70	34	6.44	10	50
F. semitectum	3	0.84	2	10	3	0.57	3	15
F. solani	16	4.47	7	35	27	5.11	7	35
F. sporotrichioides					1	0.19	1	5
F. subglutinans	2	0.56	2	10	7	1.33	4	20
F. verticillioides	33	9.22	7	25	28	5.30	9	45
Nigrospora oryzae					2	0.38	2	10
Paecilomyces variotii	1	0.28	1	5		0.00		
Penicillium	11	3.07	7	35	8	1.52	6	30
P. aurantiogriseum					1	0.19	1	5
P. ducluxii	6	1.68	3	15	3	0.57	2	10
P. digitatum	1	0.28	1	5	1	0.19	1	5
P. griseofulvum	1	0.28	1	5				
P. oxalicum	2	0.56	2	10	2	0.38	1	5
P. purprogenum					1	0.19	1	5
P. verrucosum	1	0.28	1	5				
Rhizopus oryzae					1	0.19	1	5
Stachybotrys chartarum	1	0.28	1	5	1	0.19	1	5
Stemphyllium botryosum	2	0.56	2	10				
Scopulariopsis	1	0.28	1	5	4	0.76	2	10
S. brevicaulis					1	0.19	1	5
S. brumptii	1	0.28	1	5	3	0.57	2	10
Scytalidium lignicola					1	0.19	1	5
Sterile hyphae	2	0.56	2	10	4	0.76	2	10
Sterile yellow hyphae	1	0.28	1	5				
Sterile dark hyphae	1	0.28	1	5	4	0.76	2	10
Total count	358	100	20	100	528	100	20	100

CFUs= Colony forming units /100 plates in all seasons, % CFUs= calculated to total counts, FO= Frequency of occurrence, O%= Occurrence % = (No. of isolates / Total no. of isolates) * 100.

Table (3): Inhibitory effects of three concentrations (1, 5 and 10 g/l) of clove and cumin on the growth of toxigenic fungi on PSA medium at 28 ºC.

Toxigenic fungal isolates / Spices	Diameter of the developed colonies (cm)			
	A. flavus AUMC 16585	Inhibition %	*A. flavus* AUMC16586	Inhibition %
Control (0 g/l)	4.7	0	5.0	0
Clove (1 g/l)	3.2	32	2.8	44
Clove (5 g/l)	0.6	88	Zero	100
Clove (10 g/l)	Zero	100	Zero	100
Cumin (1 g/l)	4.4	7	4.7	6
Cumin (5 g/l)	4.3	9	4.5	10
Cumin (10 g/l)	4.2	11	4.3	14

Table (4): Inhibition zone (mm) of three concentrations (1, 5 and 10 ml/l) of each of two essential oils (clove & thyme) on the growth of toxigenic fungi on PSA medium after 6 days at 28 ºC.

Toxigenic fungal isolates / Spices	Inhibition zone (mm)	
	A. flavus AUMC16585 mm	*A. flavus* AUMC16586 mm
Control (0 ml)	0	0
Clove oil (1 ml)	2.0	3.0
Clove oil (5 ml)	8.0	11.0
Clove oil (10 ml)	42.0	35.0
Thyme oil (1 ml)	1.0	2.0
Thyme oil (5 ml)	2.0	3.0
Thyme oil (10 ml)	4.0	5.0

CONCLUSION

In conclusion, the obtained results confirm that the popcorn grains were contaminated by several fungal species and some of these species have the abilities to produce mycotoxins specially aflatoxin. Milled clove buds in addition to essential oils of clove and thyme proved to be effective against the contaminating fungi of popcorn leading to partial or complete inhibition of the fungal growth and mycotoxin production. So, it is advised to use the tested natural materials for preservation of different foodstuffs.

REFERENCES

[1] I. A.I. El-Gazzar "Evaluation of New Popcorn Hybrids under Two Plant Densities". J. of Plant Production, Mansoura Univ., 12(8) (2021): 881- 885.

[2] H.E. Mosa, A.A. Motawei, M.A.G. Khalil, I.A.I. El-Gazzar, M.A.A. Hassan, S.M. Abo El-Haress & Y.A. Galal "Selection of new popcorn hybrids under two plant densities" Egypt.J. Plant Breed. 23(4) (2019): 653– 665

[3] Popcorn Board (2009): Industry Facts. www.popcorn.org

[4] P.F. Dowd & E.T. Johnson "Field incidence of mycotoxins in commercial popcorn and potential environmental influences". Mycotoxin Res. 26(1) (2010): 15-22. DOI: 10.1007/s12550-009-0036-9.

[5] H.P. van Egmond, & M.A. Jonker "Worldwide regulations on aflatoxins". In Aflatoxin and Food Safety; Abbas, H.K., Ed.; Taylor and Francis Group, LLC: New York, NY, USA, (2005): 77-93. 10.1201/9781420028171.ch5

[6] C. Zhang, Z.Qu, J. Hou & Y.Yao "Contamination and Control of Mycotoxins in Grain and Oil Crops". Microorganisms, 12(3) (2024): 1-567. https:// doi.org/10.3390/microorganisms

[7] J. Köhl, J. Postma, P. Nicot, M. Ruocco & B. Blum "Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria". Biological Control, 57(1) (2011): 1-12.

[8] R. R. Sharma, D. Singh & R. Singh "Biological control of postharvest diseases of fruits and vegetables by microbial antagonists ": A review. Biological Control, 50(3) (2009): 205-221.

[9] L.R. Beuchat "Antimicrobial properties of spices and their essential oils". In: Dillon, Y.M., Board, R.G. (Eds.), Natural Antimicrobial Systems and Food Preservation. CAB International, Oxon, (1994): 167–179.

[10] H.G. Cutler "Natural product flavor compounds as potential antimicrobials, insecticides, and medicinals". Agro-Food-Industry Hi-Tech 6, (1995): 19–23.

[11] S.S. Rathore, S.N. Saxena & B. Singh "Potential health benefits of major seed spices". International Journal of Seed Spices, 3(2) (2013): 1-12.

[12] L.R. Beuchat & D.A.Golden "Antimicrobials occurring naturally in foods". Food Technology, 43 (1989): 134–142.

[13] E.J. Smid, & L.G.M. Gorris "Natural antimicrobials for food preservation". In: Rahman, M.S. (Ed.), Handbook of Food Preservation. Marcel Dekker, New York, (1999): 285–308.

[14] A.A. Zohri, S.M. Saber, M.S. Youssef & M. Marwa Abdel-Kareem "Isolation and identification of toxigenic fungi from foodstuffs at Sohag Governorate, Egypt". Journal of Environmental Studies, 13 (2014a): 1 – 12.

[15] A.A. Zohri, S.M. Saber, M.S. Youssef & M. Marwa Abdel-Kareem "Biocontrol of some toxigenic fungal strains and their toxins production using some spices" Journal of Environmental Studies, 13 (2014b): 13 – 20.

[16] A.K. Pandey, P. Kumar, P. Singh, N.N. Tripathi, & V.K. Bajpai "Essential oils: sources of antimicrobials and food preservatives". Front. Microbiol. 7, 2161. (2017): 1- 14 DOI: 10.3389/fmicb.2016.02161

[17] H.Y. Chee and M.H. Lee (2007): "Antifungal Activity of Clove Essential Oil and its Volatile Vapour Against Dermatophytic Fungi". Mycobiology. 2007 Dec: 35(4): 241–243

[18] D. Kalemba, & A. Kunicka "Antibacterial and antifungal properties of essential oils". Current Medicinal Chemistry 10, (2003): 813–829.

[19] Association of Official Analytical Chemists (AOAC) "Official Methods of Analysis". 15th ed., Washington D.C., U.S.A. (1990)

[20] D.A. King, A.D. Hocking & J.I. Pitt "Dichloran-rose bengal medium for enumeration and isolation of molds from foods". Appl. Environm. Microbiol. 37, (1979): 959- 964.

[21] N.I. Smith & V.T. Dawson "The bacteriostatic action of rose Bengal in media used for the plate counts of fungi". Soil Sci. 58, (1944): 467-471.

[22] Y. Al-Doory "Laboratory Medical Mycology". Lea Febiger Philadelphia Kimpton Publishers, London. 240, (1980): 357-367

[23] A.D. Hocking & J.I. Pitt "Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods". Appl. Environ. Microbiol. 39(3), (1980): 488-492.

[24] B. Flannigan "Enumeration of fungi and assay for ability to degrade structural and storage components of grain". In Biodeterioration Investigation Techniques**,** ed. A. H. Waiters. Applied Science, London, (1977): 185-199.

[25] L.E. Johnson, A. Eiroy & Curl "Methods for research on the ecology of soil-borne pathogens". Minneapolis, MN 55415: Burgress publishing Company, (1972): 1- 426.

[26] C. Booth "*Fusarium* Laboratory Guide to the Identification of the Major Species". 1st End. Common, Mycol. Inst., Kew, Surrey, England, (1977): 1- 58.

[27] M.B. Ellis (1971): "Dematiaceous Hyphomycetes". Commonwealth Mycological Institute, Kew, (1971): 1- 608

[28] M.B. Ellis "More Dematiaceous hyphomycetes". The Commonwealth Mycological Institute, England, (1976): 1- 507.

[29] J.I. Pitt "The genus *Penicillium* and its Teleomorphic states". Academic Press London, (1979): 1-634.

[30] K.H. Domsch, W. Gams & T.H. Anderson "Compendium of soil fungi". IHW-Verlag, Eching, (2007): 1-672.

[31] A.H. Moubasher "Soil fungi in Qatar and other Arab countries". The Scientific and Applied Research Center, University of Qatar, Doha, (1993): 1- 566.

[32] G.S. de Hoog, J. Guarro, J. Gene & M.J. Figueras "Atlas of clinical fungi". Centraalbureau voor Schimmelcultures/ Universitat Rovira i Virgili, (2000): 1- 1126.

[33] R.A. Samson & J.C. Frisvad "*Penicillium* subgenus Penicillium: new taxonomic schemes, mycotoxins and other extrolites". Studies in Mycology (Utrecht) 49: (2004): 1– 157.

[34] J.F. Leslie, B.A. Summerell "The *Fusarium* Laboratory Manual". 1st Ed. Oxford, UK. Blackwell Publishing Ltd. (2006): 1- 387. ISBN:9780470278376.

[35] E.G. Simmons "*Alternaria*: an identification manual". CBS Fungal Biodiversity Centre, Utrecht, the Netherlands. (2007): 1- 61.

[36] R.A. Samson, J. Houbraken, U. Thrane, J. C. Frisvad, B. Andersen "Food and Indoor Fungi". CBS/KNAW/Fungal Biodiversity Centre, Utrecht, (2010): 1- 481.

[37] M.A. Ismail, S.I. Abdel-Hafez, N.A. Hussein & N. A. Abdel-Hameed "Contributions to the genus Fusarium in Egypt with dichotomous keys for identification of species". 1st.ed. Tmkarpiński Publisher Suchy Las, Poland. Disponible en línea, (2015): 1- 179 (http://creativecommons.org/licenses/by-nc/4.0/)

[38] R. Hamdy, M. M.N. El- Rify, A.A. Zohri & W.S.M. Ragab "Mycobiota and Mycotoxins Associated with Wheat Grains and some of its Products in Upper Egypt. Assiut" J. Agric. Sci., 51 (2), (2020): 122-134.

[39] N.H. Aziz, Y.A. Youssef, M.Z. El-Fouly & L.A. Moussa "Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins". Bot. Bull. Acad. Sin. 39, (1998): 279–285.

[40] I.A. El-Kady, & M.H. Moubasher "Toxigenicity and toxin of *Stachybotrys chartarum* isolates from wheat straw samples in Egyp"t. Experimental Mycology 6: (1982):25 – 31.

[41] P.M. Scott, J.W. Lawrence, & W. Van Walbeek "Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts". Appl. Microbiol. 2, (1970): 839–842.

[42] C.E. Ficker, J. T. Arnason, P. S. Vindas, L. P Alvarez., K. Akpogana, M. Gbeasor, C. De Souza & M. L. Smith "Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts". Mycoses 46 (1-2) (2003) 29-37.

[43] A. Gimeno "Thin layer chromatographic determination of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxyscirpenol, penicillic acid, patulin, and penitrem A" Journal of the Association of Official Analytical Chemists, 62: (3) (1979): 579- 585.

[44] L. Alborch, M.R. Bragulat, G. Castella, M.L. Abarca, & F.J. Cabane "Mycobiota and mycotoxin contamination of maize flours and popcorn kernels for human consumption commercialized in Spain". Food Microbiology, 32(1) (2012): 97–103.

[45] V. Krnjaja, M. Lukić, N. Delić, Z. Tomić, V. Mandić, Z. Bijelić, & M. Gogić "Mycobiota and mycotoxins in freshly harvested and stored maize". Biotechnol. Anim. Husb. 31 (2) (2015): 291–302.

[46] A.L.E. Mahmoud, A.H. Abdel-aziz & E.A. Hassan "Prevalence extent of aflatoxigenic fungi and their toxins level in corn and cornbased products". Assiut University Journal of Multidisciplinary Scientific Research. 51(1) (2022): 1–20.

[47] M.B. Roige´, S.M. Aranguren, M.B Riccio, S. Pereyra, A.L. Soraci & M.O. Tapia "Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina". Rev Iberoam Micol. 26 (4) (2009): 233–237.

[48] A. Noah, A. Hassan, M.M. Youssef, & A.A. El-Banna "Mycotoxin and Toxigenic Fungi Occurrence in Egyptian Maize". International Journal of Advanced Research 2, (2) (2014): 521-532.

[49] I. A. E1-Kady, S.I.I. Abdel-Hafez & S.S. E1-Maraghy "Contribution to the fungal flora of cereal grains in Egypt" Mycopathologia 77 (1982): 103-109.

[50] T.O. Adejumo, U.Hettwer & P.Karlovsky "Occurrence of *Fusarium* species and trichothecenes in Nigerian maize" International Journal of Food Microbiology, 116: (2007): 350–357,.

[51] J. Kos, J. Lević, O. Đuragić, B. Kokić, & I. Miladinović "Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia". Food Control. 38 (2014): 41-46.

[52] M.M.M. Deabes, H. Amra, E. Damaty & G. Rowayshed "Natural Co-Occurrence of Aflatoxins, Cyclopiazonic Acid, and their Production by *Aspergillus Flavus* Isolates from Corn Grown in Egypt". Adv. Clin. Toxicol. 3 (2018): 1–10.

[53] A.M. Moharram, M.M. Yasser, M.A. Sayed, O.A. Omar & M.M.M. Idres "Mycobiota and Mycotoxins Contaminating Rice Grains in El-Minia, Governorate, Egypt" Biosciences Biotechnology Research Asia, March, 16 (1) (2019). 167-178

[54] F.M. Bokhari "Spices mycobiota and mycotoxins available in Saudi Arabia and their abilities to inhibit growth of some toxigenic fungi" Mycobiology, 35(2) (2007): 47-53.

[55] S. Cosentino, C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi, & F. Palmas "*In-vitro* antimicrobial activity and chemical composition of Sardinian Thymus essential oils". Lett. Appl. Microbiol. 29 (1999): 130–135.

[56] H.J.D. Domans & S.G. Deans "Antimicrobial agents from plants: antibacterial activity of plant volatiles oils". Journal of Applied Microbiology, 88 (2000): 308-316.

[57] C.A. Ristori, M.S. Pereira, & D.S. Gelli "O efeito da pimenta do reino moída frente a contaminação in vitro com Salmonella rubislaw. Revista do Instituto Adolfo" Lutz, 61, (2002): 131-133.

[58] S.S. Radhakrishanan & R.R. Velusamy "Antifungal activity of some essential oils" Journal of Agriculture and Food Chemistry 51 (2003): 7596-7599.

[59] E.L. Souza, T.L. Montenegro, E. Lima, V.N. Trajano & J.M. Barbosa "Antimicrobial Effectiveness of Spices: an Approach for Use in Food Conservation Systems" Braz. Arch. Biol. Techn. 48 (4) (2005): 549-558.

[60] F. Bokhari and M.M Aly "Trials towards reduction of fungal growth and aflatoxin G_1 production in Arabic coffee using different additives" African Journal of Food Science, 3(3) (2009): 068-076.

[61] R.T. Awuah & W.O. Ellis "Effects of some groundnut packaging methods and protection with *Ocimum* and *Syzygium* powders on kernel infection by fungi" *Mycopathologia* 154 (2002): 29-36.

. [62] S. Juglal, R. Govinden & B. Odhav "Spice oils for the control of co-occurring mycotoxin producing fungi. Journal of Food Protection 65(2002): 683–687.

[63] E. Eman-Abdeen & E.M. El-Diasty "Antifungal activity of clove oil on dermatophytes and other fungi" International Journal of Advanced Research, 3 (12) (2015): 1299 – 1305.

[64] R.S. Farag, Z.Y. Daw, F.M. Hewedi & G.S.A. El-Baroty "Antimicrobial Activity of Some Egyptian Spice Essential Oils" Journal of Food Protection" Sep, 52 (9) (1989): 665-667. doi: 10.4315/0362-028X-52.9.665.

[65] S. Dubey, P.D. Upadhyay & S.C. Tripathi "Fungitoxic and insect repellant efficacy of some spices" Indian Phytopathol. 44: (1991): 101-105.

[66] E.M. Mostafa "Mycoflora and mycotoxins of some spices" M. Sc. Thesis (1990): Botany Dept., Faculty of Science, Assiut University, Egypt.

[67] A. Banso, S. Dachi, A. E. Ajewole, N. Etsu-Musa, & M. A. Ajayi "Impact of spices and aromatic plants on the growth and mycotoxin production in fungi" Journal of Current Opinion in Crop Science, 5: (1) (2024): 1-5.