Diyala Journal for Pure Science

Isolation and Identification of *Aspergillus* Species in Some Types of Spices

Nagham Saddon Ibrahim

Biology Department – Education College for Pure Science – University of Diyala

Naghamsa3doon@gmail.com

Received: 4 June 2019 **Accepted: 18 June 2019**

DOI: https://dx.doi.org/10.24237/djps.16.01.512A

Abstract

This study was carried out with the aim of examining the fungal contamination and identifying *Aspergillus* species. Five spices were collected from the local market at the district of Khalis. The spices were turmeric (*Curcuma longa*), cumin (*Cuminum cyminum*), rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum verum*), and ginger (*Zingiber officinale*). The findings showed that the samples of ginger were the most contaminated with fungi. It recorded the highest value of total fungal counts and the highest number of fungal isolates which were (3.33×10^{-3} and 20 fungal isolates). As for turmeric and cinnamon, they recorded the lowest level of fungal contamination as the total fungal count and number of fungal isolates were (0.33×10^{-3} and 2 fungal isolates).

Likewise, out of 61 fungal isolates 29 isolates were identified as *Aspergillus*. Namely, the species were 25 of *A.niger* isolates, 2 isolates for each of *A.terreus* and *A.ochracus*. However, *A. niger* recorded the highest frequency and prevalence compared with the rest of the isolates of the same species which were (86.2 and 60 %), respectively.

Keywords: Spices, Fungal Isolates, Contaminations. *Aspergillus.*

Diyala Journal for Pure Science

Isolation and Identification of *Aspergillus* Species in Some Types of Spices

Nagham Saddon Ibrahim

عزل وتشخيص انواع من فطر الـ *Aspergillus* في بعض أنواع التوابل

نغم سعدون ابراهيم

قسم علوم الحياة – كلية التربية للعلوم الصرفة – جامعة ديالى

الخلاصة

نفذت هذه الدراسة بهدف الكشف عن تلوث بعض انواع التوابل بالفطريات وتحديد انواع الفطر نفذت هذه الدراسة. تم جمع خمس انواع من التوابل من السوق المحلية لقضاء الخالص وتضمنت الكركم، الكمون، اكليل الجبل، الدارسين و الزنجبيل. بينت النتائج ان عينات الزنجبيل كانت الاكثر تلوثا بالفطريات اذا سجلت اعلى قيمة للاعداد الكلية الفطرية واعلى قيمة لعدد العزلات الفطرية وكانت (3.33×10^{-3} و 20 عزلة فطرية) اما الكركم والدارسين فقد سجلا اقل مستوى للتلوث بالفطريات اذ كانت الاعداد الكلية للفطريات وعدد العزلات الفطرية (0.33×10^{-3} و 2 عزلة فطرية) لكل منهما. كما تم خلال هذه الدراسة عزل 61 عزلة فطرية تعود 29 عزلة منها الى انواع الفطر *Aspergillus* وهي *A. niger* 25 عزلة *A. terreus* 2 عزلة و *A.ochracus* وكانت 2 عزلة. وسجل الفطر *A. niger* اعلى نسبة للتردد والظهور مقارنة ببقية عزلات الجنس ذاته وكانت (86.2 و 60) % على التوالي .

الكلمات المفتاحية: توابل، عزل فطريات، *Aspergillus*، تلوث.

Introduction

Nowadays, spices and medicinal herbs have an important role in our daily life. Spices are classified according to the International Organization for Standardization (ISO) as natural plant products which are used either as a whole or grinded. Spices are characterized by the distinctive odor and taste, as well as their color. Moreover, they are used to add flavor to the food and also for several medical purposes [1].

Spice plants often descend from the Mediterranean region, especially the spices descending from the family of *labiatae* and *Apicaceae* [2]. The spices of the tropical regions are often characterized by its acridness like rosemary, or its high aromatic content such as cinnamon, or a mixture of both, as in ginger [3]. There are many benefits to the use of spices. In addition to giving flavor to food, spices are used as preservatives, to overflow undesired smell, facilitate

digestion, and increase the individual's desire to eat spicy food [4]. However, it is more likely for spices to be contaminated with microorganisms, specifically fungi. Typically, spices may be contaminated during and before or after the harvest. Moreover, contamination may occur during storage, distribution, sale or usage process [5].

In a study conducted by [6] on spices samples included ginger and cumin, the study confirmed the presence of contamination in the studied samples with fungal and the most predominant fungal genera encountered were *Aspergillus, Penicillium, and Rhizopus.* Forty-four spice samples, including ginger, cumin and cinnamon were collected from four popular markets in Algeria to investigate their Fungal contamination. The results showed that the commonly isolated fungi were species of *Aspergillus*, *Penicillium, Mucor* and *Eurotium* [7].

Numerous isolates of *Aspergillus* and *Penicillium* were detected in spices samples obtained from Chinese markets were tested for their mould profile. The results showed that the highest fungal contamination had been obtained from cumin and pricklyash peel samples [8]. Moreover, rosmary contamination by toxigenic molds and mycotoxins was detected by [9]. The research group found that rosemary showed contamination with aflatoxin B1 and there was a positive correlation between the rosemary contamination with the presence of *A. flavus*.
Spices contamination with fungi and their toxins is a problem that threatens the health of the public, particularly in developing countries that lack the conditions of proper food storage. Public health can be highly affected by mycotoxins which may cause kidney toxicity, inhibition of immunity, mutilation of fetuses, liver diseases disruption of the central nervous system, heart, blood vessels, pulmonary systems and even death [10, 11 and 12]. Noticeably, the main source of contamination is the air. The majority of fungi spread their reproductive structures through the air which contaminate the spices either in the field or during storage [13].

Toma & Abdulla (2014) [14] presented that *Aspergillus* is one of the common species in different types of spices in the Erbil city. Similar studies have shown that many types of spices sold in the local markets were contaminated with fungi. Among them is the study of [15] which have indicated the contamination of some species of spices, including cinnamon and cumin.

The fungi found in these herbs were *Aspergillus flavus, A. terreus, A. niger, A. fumigates, Candida albicans, Rhizopus spp., Mucor spp., and Alternaria alternatia*.

Temu (2016) [16] showed 7 fungal species in 16 types of spices: *Aspergillus, Fusarium, Rhizomucor, Rhizopus, Lichtheimia, Cladosporium,* and *Penicillium.*

For the importance of spices in our daily life, the current study aimed to investigate the fungal contamination in five species of spices.

Materials and Methods

The current study was carried out in the fungi laboratory of the Biology Department / College of Education for Pure Sciences / Diyala University to detect the fungal contamination of some spices including turmeric (Curcuma longa), cumin (Cuminum cyminum), rosemary (Rosmarinus officinalis), cinnamon (Cinnamomum verum), and ginger (Zingiber officinale). which were obtained from the local market of Al-Khalis city. The study included the following steps:

Sample Collection

The table 1 was shown the type of sample of spices and the part of plant which was used in this study. Each sample was placed in black plastic bags and the information of the place and time collection was recorded. The samples transported to the laboratory for microbial analysis.

Table 1: The types of sample of spices and the part of plant included in the study

Common Name	Scientific Name	Family	The Studied part	NO. of Samples
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizomes	2
Cumin	*Cumin cyminum*	Apicaceae	Dried fruits	2
Rosemary	*Rosmarinus officinalis*	Lamiaceae	Leaves	2
Cinnamon	*Cinnamomun verum*	Lauraceae	Bark	2
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizomes	2

Isolating and Identification of Fungi from Some Species of Spices

The samples were sterilized with sodium hypochlorite solution with certain concentration of 1% for 1-2 minutes, then washed with sterilized distilled water three times from 1-2 minutes. Filter papers were used to get rid of the extra water on the samples. The sterilized samples were transferred with a sterilized forceps to petri dishes containing Potato Dextrose Agar medium (PDA medium) with the average of three pieces for each dish (in triplicate). The dishes were incubated at 25 ° C for 5 to7 days until the fungal colonies appeared. To identify fungal isolates, a portion of the growing fungal colonies on the PDA medium were moved to new dishes. The identification was based on the morphological and microscopical characteristics of the fungus according to [17, 18 and 19].

Detect of Fungal Contamination in Some Species of Spices

1. Calculating the average of the colonies forming unit (CFU):

The average of the numbers of the colony- forming unites (CFU) was calculated for each studied sample using the following formula:

$$\text{Average cfu / gram sample} = \text{number of colonies} \times \text{reversed dilution}$$

2. $$\%\ \textbf{frequency of Genus} = \frac{\text{Number of genus appearance in the sample}}{\text{total number of the genera appearance}} \times 100$$

3. $$\%\ \textbf{frequency of species} = \frac{\text{Number of species appearance in the sample}}{\text{total number of the species appearance}} \times 100$$

4. $$\%\ \textbf{frequency of Genus} = \frac{\text{colonies number of genus}}{\text{total number of genera colonies}} \times 100$$

5. $$\%\ \textbf{frequency of species} = \frac{\text{colonies number of species}}{\text{total number of species colonies [20]}} \times 100$$

Results and Discussion

Aspergillus species are the commonest in the environments [21]. Significantly, it has the ability to produce wide range of enzymes that dissolve many organic substances when grows upon. Additionally, it produces a large number of asexuale reproduction units which are called conidia. These structures could spread through air which is mostly contaminated by this species.

All the above justifies its existence on all the spice samples. The findings of current study showed that the samples of ginger were the most contaminated with fungi. It recorded the highest value of total fungal counts and the highest number of fungal isolates which were (3.33 × 10-3 and 20 fungal isolates). As for turmeric and cinnamon, they recorded the lowest level of fungal contamination as the total fungal count and number of fungal isolates were (0.33 × 10-3 and 2 fungal isolates).

Likewise, out of 61 fungal isolates 29 isolates were identified as *Aspergillus*. Namely, the species were 25 of *A.niger* isolates, 2 isolates for each of *A.terreus* and *A.ochracus*. However, *A. niger* recorded the highest frequency and prevalence compared with the rest of the isolates of the same species which were (86.2 and 60 %), respectively. The findings reported in the current study are consistent with those reported in studies which confirm that *Aspergillus* is one of the dominant species found in 24 types of spices in Egypt [22]. Moreover, the results presented in this study are interestingly consistent with many studies that have reported contamination of spices with various species of fungi specifically the *Aspergillus* [15 and 23]

Our results were in agreement with the results found by [24] were isolated fungi from different spices samples during the investigation. *Aspergillus* was the most common genus in the tested spices. *Aspergillus flavus* and *A. Niger* were the most predominant.

The results in figure 1 showed the total counts of fungi in the spice samples. Noticeably, the results showed that ginger was highly contaminated with fungi in which the total counts of fungi were 3.33×10^{-3}. Rosemary and cumin followed ginger with a record of 3.16×10^{-3} and 3.00×10^{-3} respectively. As for both turmeric and cinnamon, were recorded the lowest level of the total numbers of fungi which were 0.33×10^{-3} for each. these results may be due to the chemical components of the plant of ginger which contain of flavonoid and saboons, as well as volatile oils which include zingiberen, cineol and sesquiterpines [25], These substances affect the functions of the fungal cell membrane due to the presence of Lipophilic compounds in their composition [26].

As for cinnamon, is its content of cinnamaldehyde, benzyl alchohol, benzaldehyde and benzoic acid which is characterized by high efficiency in inhibiting the activity of fungi [27].

Current research was consistent with the findings of [6] who investigated a range of spice samples including cumin, ginger and acacia. The research group noticed that the samples were contaminated with *A. niger*. The ginger was superior to the fungus contamination compared to other spices. Consequently, the study recommended replacing ginger in foods with other antimicrobial spices such as cloves. The results presented in figure 2 showed 61 fungal isolates from the spice samples. Ginger contaminated with the highest number of 20 isolates followed by rosemary which had 19 isolates and cumin 18 isolates. As for turmeric and cinnamon, both had the lowest number of fungal isolates (2 for each).

However, the results of our research were not consistence with the findings of [28] who collected fourteen spice samples from local markets in Doha, Qatar, during 2012 to investigate their contamination with Aspergillus and *Penicillium*. Among the spice samples that have been tested, chili powder showed the highest percentage of fungi spread, while ginger and curry samples didn't show any fungal contaminations.

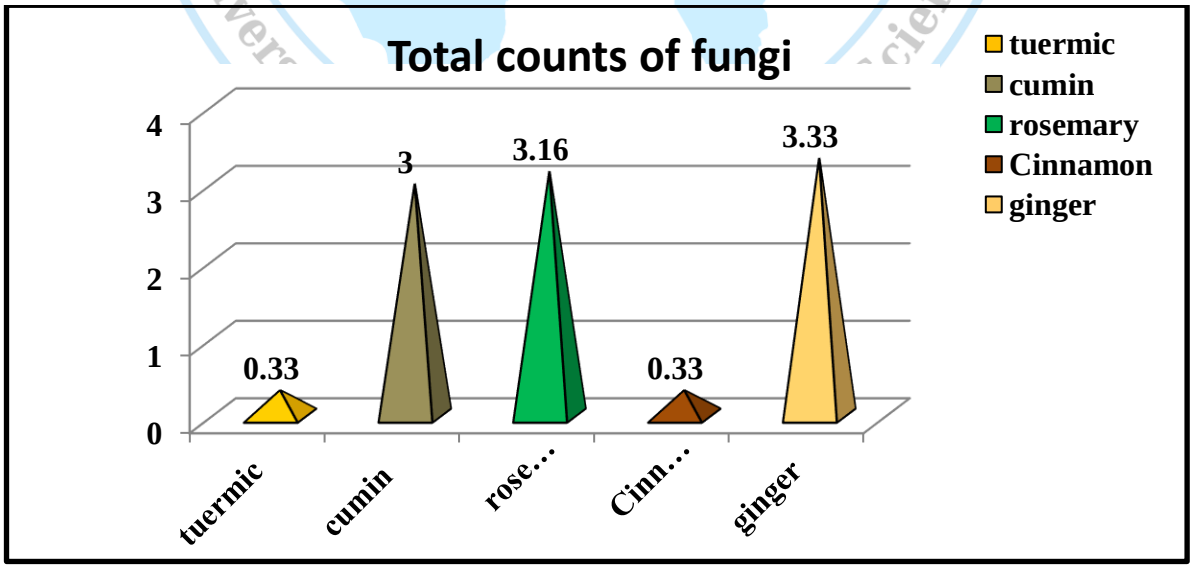

Figure 1: The total counts of fungi in some spices sold at the local market

The results presented in table 2 showed 29 fungal isolates belonging to *Aspergillus*. (with percentage 47.54 %). These isolates include 3 species of *Aspergillus:* 25 isolates of *A. niger*, 2 isolates of each of *A. terreus* and *A. ochraceus* Ahene et. al., (2011) [29] on their study that conducted on two types of spices (aniseed and rosemary) in Ghana to detect their contamination with fungus, noticed that *A. flavus, A. fumigatus, A. alutaceus, A. niger, A. sulphureus* are the most common species in the studied samples. Furthermore, the study of [23] included isolation and identification the contaminating fungi of turmeric, ginger, cinnamon and cumin. They found identificatio10 fungal isolates belonging to *Rhizopus spp.* and *Aspergillus spp*.

Concerning the frequency percentage, table 2 also showed that *A. niger* recorded the highest of 86.2% compared with the total counts of *Aspergillus* isolates followed by the other two species of *A. terreus* and *A. ochraceus.* The latters recorded frequency percentage of 6.89%. As for the frequency percentage of the three types when compared with the total counts of fungal isolates, which were 61 isolates, were 40.98, 3.27 and 3.27 % respectively. Furthermore, table 2 showed the occurrence percentage of each of the three species were *A. niger* recorded the highest occurrence percentage which was 60% followed by 40% *A. terreus* and 20% *A. ochraceus*. Hamza et al., (2013) [30] tried to identify the contaminated fungi for spices that were locally produced in Baghdad. There results indicated existence of different types of fungus which included *Rhizopus spp., Penicillium spp., Byssochlamys spp., Cladosporium spp., Alternaria spp., Paecilomyces spp., Fusarium spp*. and the highest incidence of fungi was in the species *Aspergillus niger* (92%).

One hundred twenty-four fungal isolates belonging to four different genera were recovered with *Aspergillus* and *Penicillium* as predominant fungi in cumin and pricklyash peel with an incidence of 66.1% and 15.3%, respectively, [8]. In another study conducted by [31] to investigate the presence of fungi in different spice samples including ginger and rosemary. The results indicated that *A. niger* and *A.terreus* were among the most common species found in the studied samples.

Diyala Journal for Pure Science

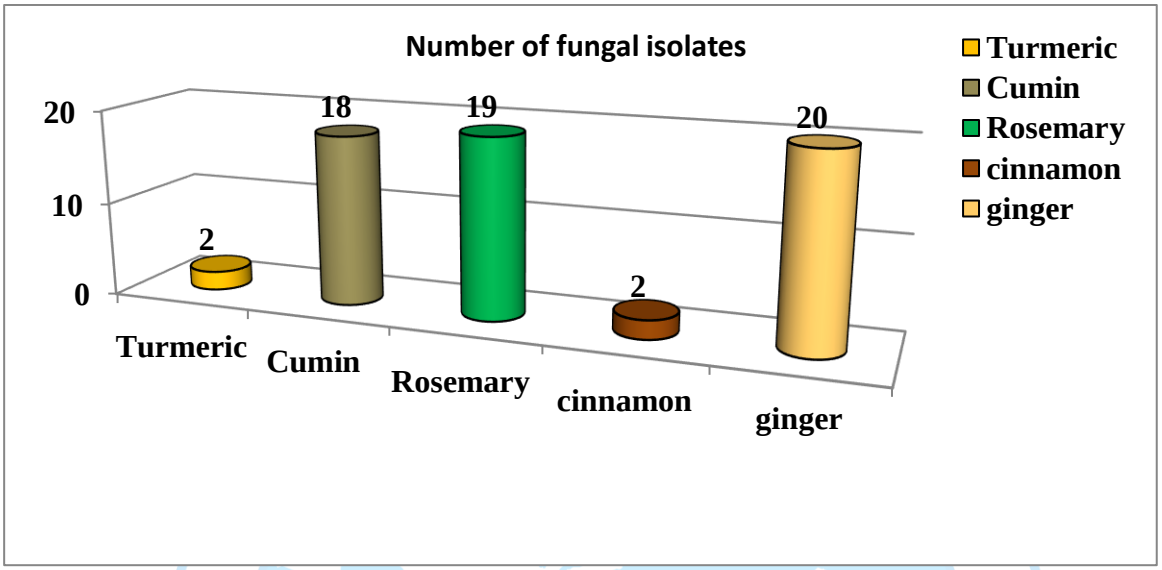

Figure 2: The counts of fungal isolates in some of the spices sold at the local market

Table 2: The number of isolates, frequency and occurrence percentage

Frequency Percentage%		Occurrence Percentage %			
Compare with *Aspergillus* species	Compare with all fungal isolates	Compare with *Aspergillus* species	Compare with all fungal isolates	No. Of isolates	Types of the *Aspergillus* species
86.2	40.98	60	60	25	*A. niger*
6.89	3.27	40	40	2	*A.terreus*
6.89	3.27	20	20	2	*A. ochraceus*
61				The total number for all fungal isolates	
29				The total number of *Aspergillus* isolates	

Conclusions

Through our findings we concluded that eating spices as a fresh form adversely affect human health, due to its high probability of contamination with fungi and bacteria. We also concluded from the results of the current research, the importance of the use of spices, which are characterized by high resistance to fungal and bacterial contamination, as it contains many active compounds that inhibit microbial activity.

References

1. K. V. Peter, Handbook of herbs and spices, 1st edition. (CRC press, Boca Raton Boston, New York Washington, DC, 2012), pp.1-3.
2. V. Heywood, M. Skoula, Perspectives on new crops and new uses, 148-151 (1999).
3. M. A. Q. F. Al-hadad, R. R. H. Al-Samarrai, M. J. Ahamish, Tikrit Journal of Pure Science ,22 (3), 89-96 (2017).
4. A. A. Arafa, Morphology of Spices, 1st edition. (Modern Library. Mansoura, 2004).
5. L. H. McKee, LWT-Food Science and Technology, 28 (1), 1-11 (1995).
6. M. Hashem, S. Alamri, Saudi Journal of Biological Sciences, 17 (2), 167-175 (2010).
7. N. Azzoune, S. Mokrane, A. Riba, N. Bouras, C. Verheecke, N. Sabaou, F. Mathieu, Quality Assurance and Safety of Crops & Foods, 8 (1), 137 – 144 (2016).
8. W. Kong, R. Wei, A. F. Logrieco, J. Wei, J. Wen, X. Xiao, M. Yang, Food chemistry, 146, 320- 326 (2014).
9. M. V. Garcia, C. A. Mallmann, M. V. Copetti, Food research International, 106, 136-140 (2018).
10. R. V. Bhat, S. Vasanthi, Food safety in food security and food trade: mycotoxin food safety risk in developing countries. (Washington D.C. International Food Policy Research Institute, 2003).
11. G. S. Shephard, Food Additives and contaminants, 25 (2), 146-151 (2008).
12. C. P. Wild, Y. Y. Gong, Carcinogenesis ,31 (1), 71-82 (2009).
13. L. Weaver, H. T. Michels, C. W. Keevil, Letters in applied microbiology, 50 (1), 18-23 (2010).
14. F. M. Toma, Q. F. Abdulla, Research Journal of Environmental and Earth Sciences, 5 (3), 131-138 (2013).
15. A. Abdul-Elah, Z. Shehab, D. Abdul Karim, Iraqi Journal of Market Research and Consumer Protection, 3 (6), 1-10 (2011).
16. G. E. Temu, Journal of Advances in Biology and Biotechnology,8 (2), 1-8 (2016).

17. M. A. Klich, Identification of common *Aspergillus* species (Utrecht, Netherlands: Centraalbureau voor Schimmelcultures, 2002).

18. R. A. Samson, E. S. Hoekstra, J. C. Frisvad, Introduction to Food - and Airborne Fungi, 7th edition. (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 2004)

19. D. H. Ellis, S. Davis, H. Alexiou, R. Handke, R. Bartley, Descriptions of Medical fungi. 2nd edition. Mycology unit womens and childrens hospital. (north Adelaide 5006, Australia, 2007).

20. C. J. Krebs, Ecology: The Experimental Analysis of Distribution and Abundance (Harper and Row Publisher, New York 1978).

21. N. Nolard, M. Detandt, H. Beguin, Ecology of Aspergillus species in the human environment. In *Aspergillus* and *aspergillosis* (Springer, Boston, MA, 1988), pp. 35-41.

22. I. A. E- Kady, S. S. E-Maraghy, M. Mostafa, Mycopathologia, 120, 93-101 (1992).

23. M. Melitane, A. Al-Zraidi, E. Hanish, R. Samio, M. Abdul Aali, Isolating and Defining the Fungi Associating some of the most Consumed Spices in the Local Markets in Misurata, In: Special Bulletin of the Second Annual Conference on the theories and applications of basic and vital sciences, 358 – 366 (2018).

24. F. M. Bokhari, Mycobiology, 35 (2), 47-53 (2007).

25. L. D. Kapoor, Handbook of medical plants (Crc press, Bocaraton, Florida, 1990), PP.185.

26. M. M. Cowan, Clinical microbiology reviews ,12 (4), 564-582 (1999).

27. A. A. H. Ali, S. Al Yazji, Diyala Journal of Agricultural Sciences, 26 (2), 287-300 (2010).

28. W. Hammami, S. Fiori, R. Al Thani, N. A. Kali, V. Balmas, Q. Migheli, S. Jaoua, Food Control, 37, 177-181 (2014).

29. R. E. Ahene, G. T. Odamtten, E. Owusu, African Journal of Environmental Science and Technology, 5 (9), 633-640 (2011).

30. I. Sh. Hamza, I. A. Khalaf, T. Kh. Sharif, International Journal for Sciences and Technology, 143, 1-13 (2013),

31. Q. A. Mandeel, Mycopathologia, 159 (2), 291-298 (2005).