

Research Article

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Mycoflora Detection in Some Dry Plants Used in Libyan Folk Medicine

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Abstract: laurel Bay, Guava and Senna were purchased from different herbalist shops in Al-Beyda city. Moisture content was determined, and the levels of fungal contamination were detected in all samples by incubating them on 2% water agar petri dishes and purifying on potato sucrose agar at $25 \pm 1^\circ\text{C}$. The higher moisture contents of 50% and 25% were recorded in Guava and Greek laurel Bay respectively. Seven different genera and 12 species were isolated and identified as *Alternaria alternata*, *Aspergillus flavus*, *A. carbonarius*, *A. niger*, *A. parasiticus*, *Cheatomium globosm*, *C. funicola*, *Cheatomium sp.*, *Cladosporium cladosporides*, *Phoma sp.*, *Pealosporium herbarum* and *Ulocladium charturm*. The higher diversity of 14 isolates and the higher frequency of 38.9% were found in guava following by 11 isolates and 30.6% in Greek laurel Bay. The relative density of contamination for fungi isolated revealed that *A. alternata* and *A. niger* are maximum (15.7%) followed by *C. funicola* (13.7%) and the minimum number of fungi recorded was 2% for *P. herbarium*. Screening tests for mycotoxins producibility in isolates of fungi by using vapor ammonia liquid on PSA medium, among which *A. flavus*, *A. carbonarius*, *A. niger*, showed positive results for mycotoxins production.

Keywords: Medicinal Plant; Moisture content; Fungi; Mycotoxins, Libya.

كشف الميكوفلورا في بعض النباتات الجافة المستعملة في الطب الشعبي الليبي

المستخلص: أُجريت الدراسة الحالية لبحث الفطريات المرافقة لبعض النباتات الجافة المستعملة في الطب الشعبي. جُمعت أوراق خمسة عشر عينة تعود لخمسة نباتات هي: السدر، الزريقة، الغار، الجوافة، والسناميكي تم شراؤها من محلات عطارة مختلفة في مدينة البيضاء. قُدر المحتوى الرطوبي ومستوى التلوث في العينات باستخدام الاجار المائي فيما تمت التتقية على الوسط PSA، وبينت النتائج تسجيل أعلى محتوى رطوبي 50% و 25% في أوراق الجوافة والغار على التوالي. تم تعريف سبعة أجناس فطرية ضمت 12 نوعاً مختلفاً: *Aspergillus flavus*, *Alternaria alternata*, *C. funicola*, *Cheatomium globosm*, *A. parasiticus*, *A. niger*, *A. carbonarius*, *Phoma sp.*, *Cladosporium cladosporides*, *Cheatomium sp.*, *funicola*, *Pealosporium herbarum* و *Ulocladium charturm*. سجل نبات الجوافة وجود 14 عزلة فطرية بتكرار 38.9% متبوعاً بـ 11 عزلة في نبات الغار وتكرار 30.6%. كانت أعلى كثافة 15.7% للفطريات *A. alternata* و *A. niger* متبوعاً بـ 13.7% *C. funicola* وأقل كثافة 2% لـ *P. herbarium*. اختبر قدرة العزلات على إنتاج السموم في الوسط PSA بالتعريض لأبخرة الأمونيا السائلة بين أن الأنواع *A. flavus*, *A. carbonarius* و *A. niger* مُنتجة للسموم.

الكلمات المفتاحية: نباتات طبية، المحتوى الرطوبي، الفطريات، سموم فطرية، ليبيا.



INTRODUCTION

In Libya, the use of folk medicine is one of the oldest, richest, and most diverse traditions, associated with the use of medicinal plants. Different parts of medicinal plants, like (bark, stem, leaves, root, fruit etc.) are used in crude, powered form, as well as boiled or soaked in water to treat cough and common cold, asthma, diarrhea and constipation, accelerated heart rate, and getting rid of fat, colic, and kidney stones. (Al-Kady & Ebshaina, 1988; Al-Kady, 2004). The fungi accompany medicinal plants during their growth in the field, and others contaminate plants during drying, harvesting and storage processes (Roy & Chourasia, 1989; Rashidi & Deokule, 2012).

Many reports about medicinal plants contamination with fungi were published. In a study conducted by (Shakhenib et al. 2011), were able to isolate 43 genera of fungi from *Thymus* sp., *Mentha longifolia* and *Trigonilla foenun-graecum* plants. The dominant fungi were *Aspergillus* (100%), *Alternaria* (90%), followed by *Cladosporium* spp., *Emericella*, *Ulocladium*, *Mucor* and *Cheatomium* at rates 76.66%, 73.33%, 53.33%, 56.66% and 40% respectively. In another study, (Siakrwar et al. 2014) demonstrated that *Saraca indica*, *Terminalia arjuna*, *Hemidesmus indicus* were contamination with molds such as *Alternaria*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Syncephalastrum*. Also, *A. flavus*, *A. niger*, *Mucor* spp. and *Rhizopus* spp. were dominant in some stored medicinal plants (Rawat et al., 2014).

Samples of 12 medicinal plants were collected from markets and analyzed for fungi contamination. The results showed that most of the species found belong to the genus *Cladosporium*, *Fusarium*, *Aspergillus* and *Penicillium* (Pereira et al., 2015). Fungi *A. alternata*, *A. terreus*, *C. cladospoiodes*, *Curvularia* spp., *C. globosum*, *Hansfordia sinuosae*, *Phoma multirostrata*, *Pleosporales* sp. and *Trametes versicolor* were associated with the aerial parts of *Calotropis procera*, *T. graecum*, *Vernonia amygdalina*, *Catharanthus roseus* and *Euphorbia prostrata* Sudanese medicinal plants (Khiralla et al., 2016).

In the Kingdom of Saudi Arabia, Al-Hindi et al., (2017) noted that the most commonly isolated fungal genera were in the following descending order: *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* in agarwood (Oudh). In China, a total of 126 fungi were identified from the surface of samples of medicinal herbs by morphology and ITS sequencing, with *Aspergillus* and *Penicillium* genera as the predominant contaminants (Zheng et al., 2017).

(Mbilu et al. 2018) reported that the medicinal plant *Warburgia ugandensis* was a carrier for stored fungi such as *Alternaria* sp., *A. flavus*, *Bionectria ochroleuca*, *Cladosporium* sp., *Cochliobolus sativus*, *C. acutatum*, *Diaporthe amygdali*, *Guignardia mangiferae*, *Fusarium oxysporum*, *Nigrospora oryzae*, *Phomopsis* sp., *Phyllostica gardeniicola*, *Trichoderma harzianum* and *Tricharina gilva*.

(Yodsing et al. 2018) recorded the presence of seven species of fungi: *A. candidus*, *Neosartorya fischeri*, *N. tatenoi*, *Paecilomyces herbarum*, *Phomopsis* sp., *Xylaria berteri* and *X. feejeensis* was a companion to the *casearia growiifolia* plant.

In another study, (Gaddawi et al., 2022) found many fungi, such as *A. alternata*, *Aspergillus* spp., *Fusarium oxysporum* and *Penicillium* sp. at different frequencies in alabaster, mint and anise herbal plants. Also, (Wei et al., 2023) evaluated the levels of contamination by toxigenic fungi in herbal medicines. They recorded *Aspergillus* (43.77%), *Fusarium* (5.17%) and

Cladosporium (4.46%) as the three predominant genera.

All plants and their products were exposed to contamination with fungi and their toxins during field growth, storage or selling. Different taxonomic groups of fungi were detected in medicinal plant samples collected from different regions, suggesting the *Aspergillus* and *Penicillium* groups as the most predominant genera. Many species of *Aspergillus* and *Penicillium* genera are known mycotoxin-producers (Al-Hindi et al., 2017; Zheng et al., 2017; Gaddawi, et al., 2022; and Wei et al., 2023). Many reports showed the role of relative humidity and moisture content in stored plant materials to justify contamination with mold fungi (Moreno-Romo, 1985; Roy and Chourasia, 1989; El-Gali, 1996).

The fungi caused discoloration, quality deterioration, a reduction in commercial value, and a risk to human health, especially after medication with them because some fungi produce mycotoxins in these plants that cause several ailments to the liver, kidney, nervous system, muscles, skin, etc. in low dosages (Rodricks, 1976; Rai and Mehrotra, 2005; Truckesses and Scott, 2008).

Because of the increase in the use of storage medicinal plants and obtaining them easily without any adequate conditions for their use, the present work investigated the presence of fungi in five types of medicinal plants that may be contaminated with storage mold and the rapid detection of toxins from fungal isolates.

MATERIALS AND METHODS

Source of samples

A number of 15 samples of dry medicinal plants exposed for sale were collected from different herbalist shops in Al-Beyda city, Libya. The plants listed in Table (1) were packed in polythene bags and brought to the microbiology laboratory at Agriculture College, Omer Al-Mukhtar University, for further studies.

Table (1): List of used medicinal plants

S. No	English name	Scientific name	Family
1	Greek laurel Bay	<i>Laurus nobilis</i>	Lauraceae
2	Guava	<i>Psidium guajava</i>	Myrtaceae
3	Lotos	<i>Zizyphus lotus</i>	Rhamnace
4	Senna	<i>Senna acutifolia</i>	Caesalpinaceae
5	Zoreka	<i>Globularia alypum</i>	Globulariaceae

Moisture content

To calculate the moisture content of the sample, 12 grams were weighted and placed in the oven at 100°C for 24 hours. The sample was taken out of the oven and placed in a glass desiccator until it cooled completely, then weighted again and returned to the oven for an hour. After drying, samples were weighted for three times and the moisture content was estimated according to Essono *et al.*, (2007): $MC = [(W_i - W_f) / W_i] \times 100$, where MC = moisture content, W_i = initial weight, and W_f = final weight. Therefore, the difference between the Initial and final weights equals the amount of moisture.

Isolation and identification of mycoflora:

Samples were washed with tap water to remove soil and dust particles. They were cut into seg-

ments (0.5 cm), surface sterilized with 1% sodium hypochlorite solution for 2 minutes, then rinsed three times in sterile distilled water, placed on a filter paper and left to dry. The pieces were distributed on 2%WA Petri dishes at a rate of 5 pieces per plate, and incubated at $25 \pm 1^\circ\text{C}$ for 5-7 days under 12 hours photoperiod. The fungal growth visible on the infected parts was purified dishes by hyphal tip transfer (Burgess, et al. 2008). A small agar block taken from the margin of the colony was transferred to plate on clean PSA using a flat needle. All fungi were maintained on PSA slants for further studies.

The fungi were primarily identified with the naked eye based on apparent characteristics such as colony color, existence or lack of aerial mycelia, and by lactophenol cotton blue stain for checking color and density, presence or lack of acervulus, pycnidium, and other asexual reproductive organs in cultural media, consisting of shape and size of conidia, conidiophores, pycnidia, and formation of hair in pycnidium under the compound microscopic (Malone & Muskett, 1997 ; Barnett & Hunter, 1998 ; Kidd et al., 2016).

Recorded of results

Frequency (Fr) and relative density (RD) of different fungal species can be expressed by means and calculated according to the following formulas (Girridher & Ready, 1997): $\text{Fr}(\%) = (\text{ns}/\text{N}) \times 100$ ns , number of samples in which the genus or species is detected; N – the total number of samples, $\text{RD}(\%) = (\text{ni}/\text{Ni}) \times 100$ ni, the number of isolates of the genus or species, Ni the total number of detected isolates.

Determination of toxigenic fungi:

In this experiment, a rapid detection of toxins was used. All isolates were grown on PSA medium in Petri dish at 25°C for 7 days. The dish was inverted, and 2ml of ammonium hydroxide solution 25% was placed in the plate cover, and closed by para film and returned to the incubator for 4 days to expose for ammonia vapor. Each treatment was repeated 3 times, and the dishes were monitored during this period to note and record any change in color for indicating the production of toxins. The change in culture pigments from underside to pink color indicated toxin secretion (Saito & Machida, 1999).

RESULTS

Moisture content

The moisture content observed in all medicinal plants ranged from 8% to 50%. However, the highest mean moisture content was recorded in Guava (50%) followed by Greek laurel Bay (25%) and Zoreka (16.7), while the lowest mean moisture content was observed in Senna (8%), as shown in Table (2).

Table (2): % Moisture content in different medicinal plant samples

Name of Plant	Weight (gm)		% Moisture content
	Initial weight (W_i)	Final weight (W_f)	
Greek laurel Bay	12	9	25
Guava	12	6	50
Lotos	12	11	8
Senna	12	11	8
Zoreka	12	10	16.7

Isolation results from samples showed that presence twelve species of fungi were recorded, in-

cluding: *A. alternata*, *A. flavus*, *A. carbonarius*, *A. niger*, *A. parasiticus*, *C. globosum*, *C. funicola*, *Cheatomium* sp., *C. cladosporides*, *Phoma* sp., *P. herbarum* and *U. chartum* (Fig.1).

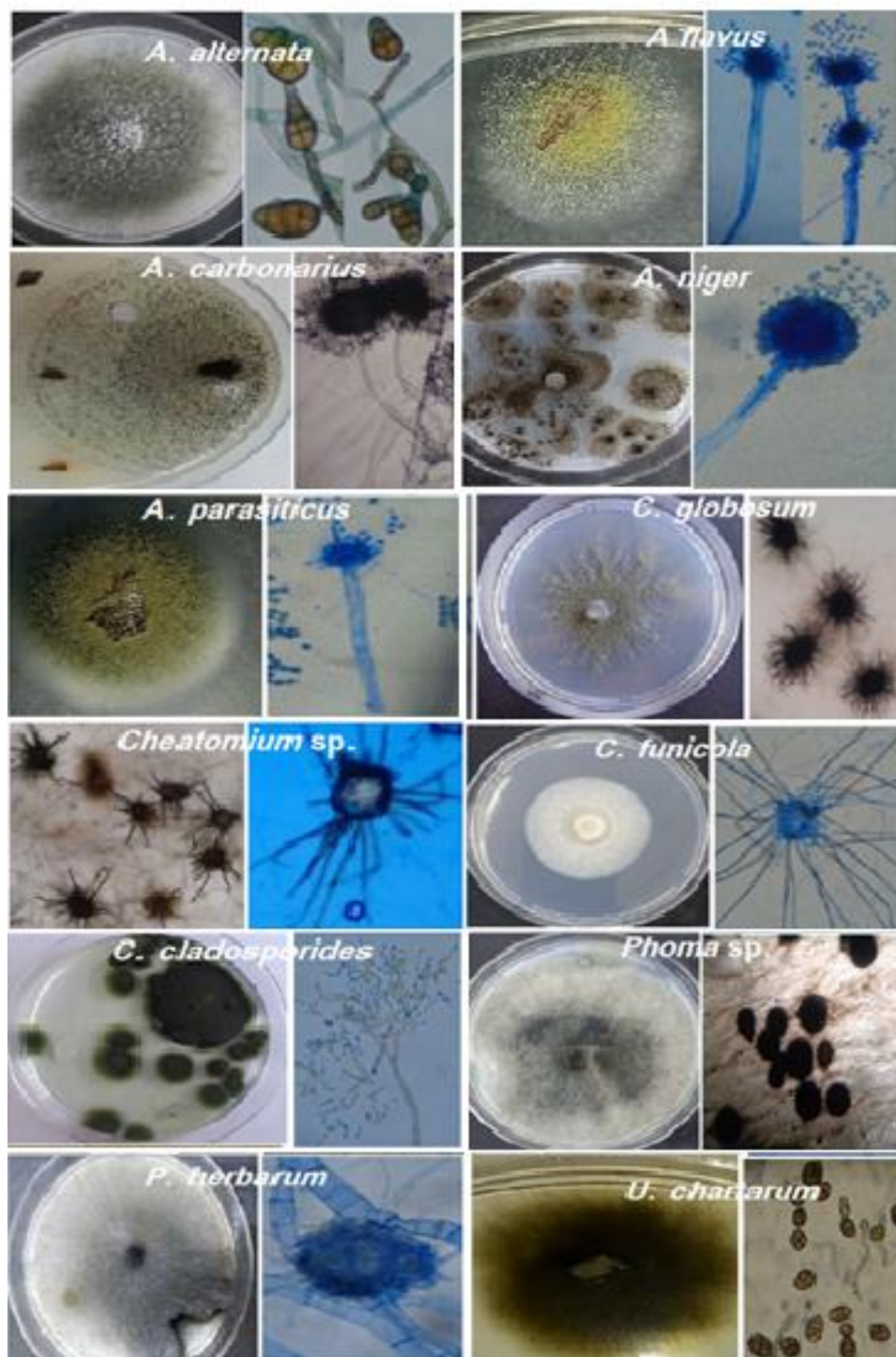


Figure: (1). Colonies of isolated fungi on PSA medium

Results in Table (3) shows the list of fungi in plant samples. Among these fungi, three species belong to *Cheatomium*, four species belong to *Aspergillus*, whereas *Alternaria*, *Cladosporium*, *Phoma*, *Pealosporium* and *Ulocladium* were appeared as one species. The highest diversity of 14 species of fungi was found in guava followed by Greek laurel Bay (11), Senna (10) and 9 in

Zoreka, and the lowest 7 species in Lotos. *Aspergillus* spp (19) and *Cheatomium* spp (14) were more commonly detected than other genera of fungi. *Alternaria*, *Aspergillus* spp., *Cheatomium* spp. were found in all examined medicinal plants, while *Cladosporium* was found in Greek laurel Bay and Lotos. *Phoma* in Zoreka and Lotos, *Ulocadium* in Greek laurel Bay and Guava. *Pealospodium* found only in Zoreka.

Table (3): Samples of medicinal plants contaminated with one or more fungal species

Name of plants	Sample No.	Name of fungi											Fungal contamination per sample	Total
		<i>A. alternata</i>	<i>A. flavus</i>	<i>A. carbonarius</i>	<i>A. niger</i>	<i>A. parasiticus</i>	<i>C. globosum</i>	<i>C. funicola</i>	<i>Cheatomium</i> sp.	<i>C. cladosporides</i>	<i>Phoma</i> sp.	<i>P. herbarium</i>	<i>U. chartum</i>	
Greek laurel Bay	1	+	-	-	+	-	-	+	+	+	-	-	+	6
	2	-	-	-	-	-	+	+	+	-	-	-	+	4
	3	-	-	-	-	-	-	-	-	-	-	-	+	1
	4	+	-	-	+	-	-	-	+	-	-	-	-	3
Zoreka	5	+	-	-	+	-	+	-	+	-	+	+	-	6
	6	-	-	-	-	-	-	-	-	-	-	-	-	0
	7	-	+	+	-	+	-	-	-	-	-	-	+	4
Guava	8	+	+	+	-	+	-	+	-	-	-	-	+	6
	9	-	+	+	-	+	-	+	-	-	-	-	-	4
	10	+	+	-	+	-	-	-	+	-	-	-	-	4
Senna	11	+	+	-	+	-	-	-	-	-	-	-	-	3
	12	+	-	-	+	-	-	+	-	-	-	-	-	3
	13	+	-	-	-	-	-	-	-	+	-	-	-	2
Lotos	14	-	-	-	+	-	-	+	-	-	-	-	-	2
	15	-	-	-	+	-	-	+	-	-	+	-	-	3
Total		8	19				14			2	2	1	5	51

Regarding the frequency of fungi, the results recorded in Table (4) indicated a difference in the frequency of fungi isolated from plants. The minimum percentage of contamination was detected in Lotos (19.4%), while the maximum contamination was in Guava (38.9%) followed by Greek laurel Bay (30.6%).

Considering the density of fungi represented in Table (5), the results showed that *A. alternata* and *A. niger* had the highest density (15.7%) followed by *C. funicola* (13.7%) while *P. herbarium* had the least density at 2%.

Determination of toxigenic fungi:

Results of the ability of fungal isolates to secrete toxins in PSA medium after being exposed to liquid ammonia vapor were recorded, showing a change in culture pigments from underside with different intensities. Among these isolates *A. flavus*, *A. carbonarius*, *A. niger*, showed positive results for mycotoxins production compared to the color before treatments. (Table 6).

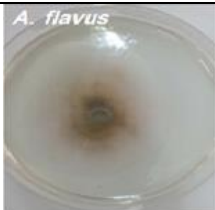
Table (4): % Frequency of fungal species isolated from different medicinal plants

fungi	Greek laurel Bay	Zoreka	Guava	Senna	Lotos
<i>A. alternata</i>	33.3	66.7	33.3	100	33.3
<i>A. flavus</i>	0	0	100	66.7	0
<i>A. carbonarius</i>	0	0	100	0	0
<i>A. niger</i>	33.3	66.7	0	100	66.7
<i>A. parasiticus</i>	0	0	100	0	0
<i>C. globosum</i>	33.3	33.3	0	0	0
<i>C. funicola</i>	66.7	0	66.7	33.3	66.7
<i>Cheatomium</i> sp.	66.7	66.7	0	33.3	0
<i>C. cladosporides</i>	33.3	0	0	0	33.3
<i>Phoma</i> sp.	0	33.3	0	0	33.3
<i>P. herbarium</i>	0	33.3	0	0	0
<i>U. charturm</i>	100	0	66.7	0	0
Mean	30.6	25	38.9	27.8	19.4

Table (5): % Relative Density of fungal species isolated from different medicinal plants

Fungal species	Number of isolates	% contamination
<i>A. alternata</i>	8	15.7
<i>A. flavus</i>	5	9.8
<i>A. carbonarius</i>	3	5.9
<i>A. niger</i>	8	15.7
<i>A. parasiticus</i>	3	5.9
<i>C. globosum</i>	2	3.9
<i>C. funicola</i>	7	13.7
<i>Cheatomium</i> sp.	5	9.8
<i>C. cladosporides</i>	3	5.9
<i>Phoma</i> sp.	2	3.9
<i>P. herbarium</i>	1	2
<i>U. charturm</i>	5	9.8
Total	51	

Table (6): Test of detection of toxins secretion in PSA solid medium

Fungi	Ammonia vapor test		Response
	Before	After	
<i>A. flavus</i>			+
<i>A. carbonarius</i>			+
<i>A. niger</i>	-	-	+
+: found			
-: not found			

DISCUSSION

During this study, we collected 15 samples of medicinal plants, and isolated 12 species belonging to 7 genera including, *A. alternata*, *A. flavus*, *A. carbonarius*, *A. niger*, *A. parasiticus*, *C. globosm*, *C. funicola*, *Cheatomium* sp., *C. cladosporides*, *Phoma* sp., *P. herbarum* and *U. char-turm*. Other similar studies have recorded the presence of such fungi on dried medicinal plants or spices (Zdenk & Stjepan, 1999; Bugno et al., 2006; Rashidi & Deokule, 2013; Gaddawi, et al., 2022; Wei et al., 2023).

Also, the results demonstrated a correlation between the highest moisture and the fungal frequency in medicinal plants. Guava plant has the highest moisture content (50%) and the highest fungal frequency (38.9%). Hale (1998) reported that the presence of moisture content in medicinal plant samples justifies the favorable impact of fungal growth on store medicinal plants. The high level of moisture content and fungal frequency may be due to the fact that most of these samples are imported, and in addition, the preventive measures were not applied during storage and exposure for the sale of these medicinal plants.

From this study, it was noticed *Aspergillus* spp. followed by *Cheatomium* spp. and *A. alternata* were isolated from all samples, they appeared to be the most predominant fungi of medicinal plants. The results agree at large with many of the investigators working on spices and medicinal plants (Sumanth et al, 2010; Shakhnib et al. 2011; Toma & Abdulla, 2013; Gaddawi, et al., 2022; Wei et al., 2023).

Aspergillus spp. recorded the highest incidence. The same result was recorded by (Chen et al. 2020) on medicinal herbs. It is attributed to its ability to produce the large number of conidia spores that widely distributed (Domsch et al., 1980) and exploit the different substances as carbon sources for nutrition and growth (Dickinson & Boardman, 1970), Also, for living at a low level of humidity (Onesirosan, 1982).

From this study, we noticed an increase in the number of fungi (Dematiaceous) that produced melanin pigments, which enhance the survival and competitive abilities of species in certain environments (Bell & Wheeler, 1986).

Cheatomium spp is one of the fungi isolated from all tested samples due to its ability for undergo cellulose analysis by cellulose enzymes and produce fruiting bodies structures to tolerate environment unsuitable conditions (Udagawa et al., 1979).

Considering mycotoxins production, the experiments recorded the ability of *A. flavus*, *A. niger*, and *A. carbonarius* to produce toxins in PSA medium after exposing to ammonium hydroxide vapor. Similar studies have demonstrated the ability of such fungi to secrete toxins (Wicklow et al., 1996; Bugno et al, 2006; Rashidi & Deokule, 2013; Pereira et al., 2015; Al-Hindi et al., 2017; Chen et al., 2020).

CONCLUSION

From the present study, it was concluded that all plants samples were contaminated with storage mold fungi and toxigenic fungi. The contamination level was related to the highest moisture content. Because medicinal plants are used in folk medicine for direct human purposes, so the contamination with fungi must be detected and their use prevented in order to preserve public health.

ETHICS

The authors should address any ethical issues that may arise after the publication of this manuscript.

Duality of interest: The authors declare that they have/ have no duality of interest associated with this manuscript.

Author contributions: The first author supervised the project. He wrote the results, while the second author did the practical experiments.

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