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# Biodiversity of Soil and Air-Borne Fungi in the Northern Border Region of Saudi Arabia

**S.K. Hemida[1,2] and M. A. Abdel-Sater[2]**

[1]Department of Biology, Faculty of Arts & Science (Rafha),
University of Northern Border, Saudi Arabia.
[2]Department of Botany & Microbiology, Faculty of Science, Assiut University, Assiut, Egypt.

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**The mycological analysis of 40 soil samples and the air at northern border region, in Saudi Arabia was studied during the period from October to December 2015. The dilution- and exposed- plate techniques and three isolation media incubated at 28°C were used for isolation of soil and air-borne fungi. Chemical analysis revealed that soil samples have very low water content and with poor total soluble salts. A total of 56 +1 variety species related to 20 genera were isolated from 40 soil samples using Cz, cellulose and 10% NaCl media incubated at 28C, while, 28 species belonging to 18 genera were encountered from the air of the four regions. In soil the hyaline fungi like *Aspergillus*, *Penicillium, Myrothecium* and *Fusarium* were predominant over darke-coloured ones. On the contrary, in the air, the dematiaceous fungi e.g. *Alternaria*, *Cladosporium*, *Ulocladium*, *Phoma*, *Stachybotrys*, *Humicola* and *Derchslera* outnumbered the hyaline ones. There was a significant difference in the occurrence and diversity of fungi recovered from soil, or air in the four studied regions. Among 6 isolates of *A. flavus* strains screened for aflatoxin production 3 could produce B1 & B2 and G1 & G2. Knowledge of species and density of soil and outdoor airborne fungi in the studied environment can be especially important in the diagnosis and treatment of various allergic diseases. This study provided some information regarding the soil and air borne fungal composition at the Northern border region of Saudi Arabia and suggesting a further investigation to correlate between the common human allergies among the population and the incidence of soil and airborne fungi in the environment of this region.**

**Keywords:** Aerobiota, outdoor, allergen, soil fungi, aflatoxins, seasonal distribution.

Soil fungi are important component of the soil habitat as ecologically their role to control the nutritional cycle to maintain the soil fertility and structure[1, 2]. If we remember that only about 6- 7% of the total fungal species on earth suggested to be 1.5 million are known[3]. Thus, mycologists ever where are strongly urged to work very hard to search for new species in different ecosystems around them. Several studies on soil fungi has been investigated from various points of view in many parts of the world[4-10].

* To whom all correspondence should be addressed.
E-mail: samehhemida@yahoo.com

The term "Air-born fungi" is namely that the spores of fungi carrying by atmosoheric air. The intensity of fungal spores increases depending on air pollutionand vary with time of day, weather, season, geographical location and flora combination[11-13]. The study of aeromycology is important in plant pathology and in disease forecasting of plant diseases. Aerobiological studies conducted in relation to respiratory allergic diseases[14]. Aeromycological researchs from the Middle East area are scattered, in Egypt[15,16], in Kuwait[17], in Qatar[18], in Saudi Arabia[14, 19], in Yemen[20], In Libya[21], in Turkey[11, 22-24], in Iran[25] , and in Jordan[26]. In the aerospora several fungal genera and species were found to be the most dominant[27,28].

Aflatoxins are a group of mycotoxins that produce by toxigenic strains of *Aspergiullus* flavus and A. *parasiticus*[29-32]and other species are also reported as AFs producers[33]. Aflatoxins contaminate different types of food and feed commodities, especially in hot and humid regions of the world[34]. The major AFs are characterized as $B_1$, $B_2$, $G_1$, and $G_2$ of which aflatoxin B is the best known because of its hepatocarcinogenic nature[35]. From golobal prospective of food safty and security, aflatoxins contamination of food has gained much attention as potential health hazard for humans and animals[36].

In Saudi Arabia, especially the northern border region, information on the fungal ecology of desert and reclaimed soil and air borne fungi is limited. Thus, the aim of the present work was designed to investigate the diversity of fungi in soil of the northern border region.Seasonal fluctuations of fungi over these regions was carred out bimonthly during October - December 2015. Also, the potentionality of the commonest fungi for aflatoxins production was evaluated.

## MATERIALS AND METHODS

**Sampling location**

This study was carried out in four cities (Rafha, Lina, Al-Uwaygilah and Arar), the Northern border region of Saudi Arabia, bordering Iraq and Jordan. Sampling was conducted bimonthly during the period from October to December 2015.

**Collection of soil samples**

A total of 40 soil samples were collected from the four governorates (10 for each). At least five samples were taken at random from each place, then the five soil samples were brought into one compsite sample which mixed thoroughly several times. Soil samples were put directly each into a clean plastic bages and brought into the laboratory and kept at 5°C till fungal analysis.

**Determination of soil moisture content (MC)**

The moisture content of soil was determined by drying replicates of freshly collected samples in an oven at 105 C till constant weight. The loss of weight was determined, and then the percentage of moisture content was calculated from the following equation:

$$\% \text{ Moisture content} = \{(A - B) \div B\} \times 100$$

Where

A = weight of sample before drying and B = weight of sample after drying.

**Determination of total soluble salts (TSS)**

The specific electrical conductance was measured in the soil extract using the conductance meter (YSI, model 35). The percentage TSS in the samples were estimated using this equation: % TSS in dry sample = 0.064 X EC X extract ratio. The conversion factor to percentage salts (0.064) fairly applied for solutions extracted from the soil[37].

**Isolation of soil fungi**

The dilution- plate method was used for enumeration of soil fungi as described by Johnson *et al*.[38]and employed by Moubasher and his collaborators. The plates (5 plates for each type of medium) were incubated at 28 C for 1- 3 weeks after which the developing fungi were counted and the number of colony forming units (CFUs) was calculated per g dry soil.

**Isolation of air-borne fungi**

Sampling was conducted bimonthly during the period from October to December 2015. Fifteen replicate plates of 9 cm diameter of the three media (Czapek's, Cellulose and 10% NaCl, 5 each) were exposed for 15 minutes at a height of 60 cm above the ground level at each of the four sites. The plates were then sealed and brought back to the laboratory and incubated at 28ºC for 7-15 days, during which, the developing colonies were counted (CFU, per 5 plates each medium), isolated and identified.

**Media used for isolation of fungi**

The three types of media used for isolation of fungi were: 1) Modified Czapek's agar of the following composition (g/l): glucose 10, sodium nitrate 3.0, potassium dihydrogen phosphate 1.0, magnesium sulphate 0.5, potassium chloride 0.5, ferrous sulphate 0.01, agar 15.0), to which rose bengal (1/15000) and chloramphenicol (25 µg/ml) were added as bacteriostatic agents; 2) Czapek's agar of which sucrose was replaced by cellulose for isolation of cellulose decomposing fungi and 3) Modified Czapek's agar medium supplemented with 10% sodium chloride was used for isolation of halophilic and halotolerant fungi.

**Identification of fungi**

The following references were used for the identification of fungal genera and species[39-45].

**Screening of aflatoxins**

six strains related to *Aspergillus flavus*, isolated in the current study, were screened for aflatoxin-producing capability according to the method of Zohri[46]**.** Cultures were observed for fluorescence under long-wave UV light (365 nm) after 7 days. The positive results were shown as blue and green flourescences.

## RESULTS AND DISCUSSION

Chemical analysis of desert soil samples collected from the four regions revealed that water content and tatal soluble salts in the tested samples were very low levels. MC in the examined samples ranging from 0.4-1.8; 0.2-1.9; 0.5-3.6; and 0.6-3.0 % in the four regions, respectively. Also, as in MC, total soluble salts were remarkably higher in Al-Uwaygilah and Arar than the other two regions ranging between 0.2- 1.0 ; and 0.4-1.2 and 0.2- 1.1 . Relatively high means MC and total TSS was found in Arar (2.04 and 0.82) followed by Al-Uwaygilah (1.76 and 0.83) (Table 1). Our results were greatly harmony with the finding of Abdel-Hafez[4] who reported that soil of Saudi Arabia were generally poor total soluble salts (0.05- 0.46 %). Gherbawy *et al*.[47] indicated that the soil moisture content is low in all habitats of Taif area. Moubasher *et al*.[48] stated that MC of cultivated, desert and salt marsh soils were higher than those in Saudi Arabia and ranged from 7.5 – 22.4; 1.5- 9.5 and 5.5-19.3%, respectively. Whereas, TSS fluctuated from low value (0.2- 1.3%) in cultivated; low or moderate values in desert (0.2- 7.6%) and high or very high in salt marsh soils (4.6-46.7%). Ismail[49] rated that MC of air dust fluctuated between 0.5- 9.0% while, TSS varied from 0.03- 31.7% and most samples contained less than 02.0% of total soluble salts.

**Fungi recovered from soil**

Using three types of media incubated at 28°C, it could isolate 56 species and one variety appertaining to 20 genera from 40 soil sample collected from 4 regions (10 each) at the Northerns border of Saudi Arabia (Table 2). The results revealed that the highest counts and number of genera and species were recorded in Arar (13 genera and 25 species) and Linah (12 and 260) followed by Al-Uwaygilah (12 and 22) and Rafha (9 and 16). 1n soil the hyaline fungi were predominant over the dark-coloured ones. It may be due to the soil is densely populated by microorganisms and competition for extence is very sever. On the other hand, fungi are relatively protected from the injurious effects of atmospheric conditions, high light intensity, and deep diurnal fluctuations of temperature and humidity. These conditions may selectively favour hyaline fungi over the dark-coloured (melanin-containing) fungi.

There was remarkably high incidence of fugal total counts in soil samples gathered from Arar (1065 conlonis/ g) and Linah (850) followed by Al- Uwaygilah (760) and Rafha (320). This may be associated with the high activities of human and animals which led to increased amount of nutrients in the remains of plant, animal and man in this area providing the optimal condations for microbial growth[50]. In this respect, Comprehensive studies on soil fungi began with work of Adametz[51]

**Table 1.** Percentage moisture content (MC%) and total soluble salts (TSS%) of the examined soil samples

| Samples | Rafha | | Linah | | Al-Uwaygilah | | Arar | |
|---|---|---|---|---|---|---|---|---|
| No. | MC% | TSS% | MC% | TSS% | MC% | TSS% | MC% | TSS% |
| 1 | 1.8 | 0.2 | 1.8 | 0.9 | 1.5 | 0.5 | 2.1 | 1.1 |
| 2 | 0.9 | 0.2 | 0.7 | 1.0 | 1.3 | 0.6 | 1.8 | 1.2 |
| 3 | 1.6 | 0.3 | 1.9 | 0.8 | 0.9 | 0.2 | 2.6 | 0.9 |
| 4 | 1.5 | 0.9 | 0.9 | 0.4 | 1.8 | 1.0 | 2.2 | 0.6 |
| 5 | 1.6 | 0.3 | 0.2 | 0.3 | 0.5 | 0.7 | 1.7 | 0.7 |
| 6 | 1.0 | 1.0 | 1.3 | 0.2 | 2.2 | 0.6 | 2.5 | 1.0 |
| 7 | 0.4 | 0.6 | 1.1 | 0.5 | 3.6 | 0.8 | 3.0 | 0.8 |
| 8 | 0.8 | 0.3 | 0.9 | 0.6 | 1.9 | 1.0 | 0.6 | 0.9 |
| 9 | 1.2 | 0.4 | 1.8 | 0.7 | 2.1 | 1.1 | 2.0 | 0.4 |
| 10 | 1.3 | 0.9 | 1.0 | 0.9 | 1.8 | 0.9 | 1.9 | 0.6 |
| Mean | 1.21 | 0.51 | 1.16 | 0.63 | 1.76 | 0.83 | 2.04 | 0.82 |

**Table 2.** Total counts (TC, calculated per g) and percentage frequency (F %, out of 10 samples) of fungal genera and species isolated from desert soil of the four regions on Czapek's- glucose (Cz), Czapek's –cellulose (Cell) and Czapek's- glucose supplemented with 10% NaCl agar media at 28°C

| Taxa* | Rafha | | | | | | LinahAl-Uwaygilah | | | | | | Arar | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cz | | Cell. | | 10 % NaCl | | Cz | | Cz | | Cell. | | 10 % NaCl | | CzCz | | Cell. | | 10 % NaCl | | | | | |
| | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F | TC | F |
| *Alternaria* | 26 | 5 | | | | | 46 | 4 | | | 4 | 2 | | | 2 | 1 | 20 | 2 | 16 | 3 | 24 | 3 | 38 | 3 |
| *A. alternata* | 16 | 3 | | | | | 20 | 1 | | | | | | | | | | | | | | | | |
| *A. chlamydospora* | | | | | | | | | | | | | | | 2 | 1 | 20 | 2 | 16 | 3 | 24 | 3 | 38 | 3 |
| *Alternaria* spp. | 10 | 2 | | | | | 26 | 4 | | | 4 | 2 | | | | | | | | | | | | |
| *Aspergillus* | 100 | 9 | 100 | 9 | | | 158 | 7 | 302 | 10 | | | 18 | 4 | 40 | 7 | 2 | 1 | 14 | 3 | 24 | 4 | 40 | 5 |
| *A. brasiliensis* | 4 | 2 | | | | | 60 | 1 | | | | | | | | | | | | | | | | |
| *A. carneus* | | | 8 | 2 | | | | | | | | | 6 | 1 | | | 2 | 1 | | | | | | |
| *A, flavus* | 4 | 2 | 6 | 3 | | | 6 | 3 | 120 | 5 | | | 2 | 1 | 2 | 1 | | | | | | | 8 | 2 |
| *A. fumigatus* | 18 | 7 | 34 | 4 | | | 56 | 5 | 90 | 6 | | | 10 | 2 | 38 | 5 | | | | | 14 | 2 | | |
| *A. ochraceus* | | | 8 | 3 | | | 10 | 1 | 22 | 2 | | | | | | | | | | | | | 10 | 1 |
| *A. terreus* | | | 38 | 3 | | | 4 | 2 | 68 | 4 | | | | | | | | | 6 | 3 | 10 | 1 | 4 | 1 |
| *Botryotrichum piluliferum* | | | | | | | | | 6 | 1 | | | 2 | 1 | 16 | 1 | | | | | | | | |
| *Cladosporium* | | | 6 | 1 | | | 20 | 4 | 2 | 1 | | | | | 4 | 1 | | | | | | | | |
| *C. cladosporioides* | | | 6 | 2 | | | 10 | 1 | | | | | | | 4 | 1 | | | | | | | | |
| *Cochliobolus* | | | | | | | | | | | | | 2 | 1 | 2 | 1 | | | 12 | 2 | 6 | 1 | | |
| *C. spicifer* | | | | | | | | | | | | | 2 | 1 | | | | | 8 | 1 | 6 | 1 | | |
| *Emericella* | 2 | 1 | 4 | 2 | | | 2 | 1 | 6 | 2 | | | | | | | | | | | | | 2 | 1 |
| *Fusarium* | 4 | 1 | | | 4 | 1 | 10 | 3 | | | 2 | 1 | 26 | 4 | 6 | 2 | | | 4 | 1 | 38 | 5 | | |
| *F. sambucinum* | | | | | | | | | | | | | 4 | 1 | 6 | 2 | | | | | 16 | 3 | | |
| *F. solani* | 4 | 1 | | | | | 6 | 3 | | | | | 16 | 3 | | | | | | | 14 | 2 | | |
| *Fusarium* sp. | | | | | 4 | 1 | | | | | 2 | 1 | | | | | | | | | | | | |
| *Humicola fusco-atra* | | | | | | | | | | | | | | | 4 | 1 | | | 8 | 2 | 2 | 1 | | |
| *Mucor* | 12 | 1 | | | | | 8 | 2 | 8 | 1 | | | | | 8 | 3 | | | 22 | 3 | 14 | 4 | | |
| *M. circinellioides* | 12 | 1 | | | | | 8 | 2 | 8 | 1 | | | | | 8 | 3 | | | 8 | 3 | 2 | 1 | | |
| *Myrothecium* | | | | | | | 2 | 1 | | | | | | | 38 | 4 | | | 28 | 3 | 72 | 4 | | |
| *M. verrucaria* | | | | | | | 2 | 1 | | | | | | | 38 | 4 | | | 14 | 2 | 30 | 2 | | |
| *Penicillium* | 30 | 3 | 28 | 7 | | | 52 | 7 | 96 | 3 | | | 116 | 9 | 2 | 1 | 4 | 2 | | | 32 | 5 | | |
| *P. chrysogenum* | 8 | 1 | 28 | 4 | | | 2 | 1 | | | | | 38 | 3 | | | 4 | 2 | | | | | | |
| *P. funiculosum* | 6 | 1 | | | | | | | | | | | | | 2 | 1 | | | | | 26 | 3 | | |
| *P. oxalicum* | 16 | 1 | | | | | | | | | | | 4 | 1 | | | | | | | 6 | 1 | | |
| *Penicillium* spp. | | | | | | | 48 | 4 | 96 | 3 | | | 50 | 3 | | | | | | | | | | |
| *Phoma* | | | 4 | | | | 28 | 10 | | | | | | | | | | | 24 | | 8 | | | |
| *P. glomerata* | | | 4 | 1 | | | 18 | 2 | | | | | | | | | | | | | | | | |
| *Stachybotrys chartarum* | | | | | | | 6 | 2 | | | | | | | 32 | 1 | | | | | | | | |
| *Ulocladium* | | | | | | | 28 | 3 | 62 | 5 | | | 106 | 7 | 102 | 6 | 202 | 8 | 113 | 10 | 92 | 8 | 398 | 7 |
| *U. atrum* | | | | | | | 28 | 3 | 32 | 2 | | | 84 | 5 | 60 | 3 | 34 | 2 | 64 | 7 | 72 | 5 | 358 | 5 |
| *U. botrytis* | | | | | | | | | 30 | 3 | | | | | 38 | 3 | 168 | 6 | 14 | 2 | 6 | 2 | 40 | 2 |
| *U. tuberculatum* | | | | | | | | | | | | | 22 | 2 | 4 | 1 | | | 35 | 3 | 14 | 2 | | |
| Total CFUs | 174 | 10 | 142 | 10 | 4 | 10 | 348 | 10 | 492 | 10 | 8 | 10 | 276 | 10 | 256 | 10 | 228 | 10 | 253 | 10 | 324 | 10 | 488 | 10 |
| No. of genera | 6 | 5 | 1 | 11 | 8 | 3 | 13 | 12 | 4 | 13 | 12 | 5 | | | | | | | | | | | | |
| No. of species+var. | 12 | 10 | 1 | 25 | 20 | 3 | 16 | 15 | 5 | 18 + 1 | 20 | 8 + 1 | | | | | | | | | | | | |
| Total genera =20 | 9 | 12 | 12 | 13 | | | | | | | | | | | | | | | | | | | | |
| Total spp. 56+1var. | 16 | 26 | 22 | 24+1var. | | | | | | | | | | | | | | | | | | | | |

*Genera and species encountered from one City were omitted.

who isolated 11 species of fungi from expermintal farm for purpose of making biochemical studies. Since that, the number of publications on soil fungi has grown significantly, such as: Azaz and Pekel[7] from soil samples taken from burnt forest land in Turkey; Szewczyk[8] on soil from young Scots pine plantations affected with root rot in Poland; Puangsombat *et al.*[9] from soil of Tha Kum-Haai; Hemida *et al.*[10], Abdel-Hafez *et al.*[52,53], Al-Khateeb[54] on soil collected from different habitats in Egypt.

Incidence of *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Ulocladium* and its

**Table 3.**Total counts (calculated/30 plates/ 20 min) of fungal genera and species isolated from the air of the four regions on Czapek's-glucose agar (Cz), Czapek's - Cellulose agar (Cell) and Czapek's with 10 % NaCl agar (10% NaCl) media at 28°C.

| Genera & Species | Rafha | | | Linah | | | Al-Uwaygilah | | | Arar | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cz | Cell. | 10 % NaCl | Cz | Cell. | 10 % NaCl | Cz | Cell. | 10 % NaCl | Cz | Cell. NaCl | 10 % |
| *Alternaria alternata* | 88 | 83 | 62 | 67 | 86 | 58 | 74 | 95 | 33 | 84 | 72 | 32 |
| *Aspergillus* | 26 | 45 | 52 | 62 | 26 | 53 | 32 | 27 | 13 | 39 | 61 | 6 |
| *A. flavipes* | 13 | - | - | 14 | - | - | 4 | 2 | 6 | 3 | - | 3 |
| *A. flavus* | 3 | 12 | - | 3 | 4 | - | 8 | 3 | - | 3 | 14 | - |
| *A. fumigatus* | 1 | 4 | 20 | 7 | 3 | 11 | 6 | - | - | 6 | 10 | 3 |
| *A. niger* | 6 | 25 | 17 | 24 | 10 | 36 | 12 | 22 | 7 | 21 | 13 | - |
| *A. terreus* | 1 | 4 | 7 | 11 | 6 | 6 | 2 | - | - | 6 | 24 | - |
| *A. ustus* | 4 | - | 8 | 3 | - | - | - | - | - | - | - | - |
| *Chaetomium globosum* | - | 2 | 3 | - | 1 | 5 | - | - | - | - | 4 | - |
| *Cladosporium* | 46 | 37 | 50 | 31 | 22 | 37 | 28 | 10 | 11 | 25 | 45 | 19 |
| *C. cladosporioides* | 43 | 37 | 48 | 31 | 22 | 37 | 24 | 10 | 11 | 25 | 34 | 19 |
| *C. herbarum* | 3 | - | 2 | - | - | - | 4 | - | - | - | 11 | - |
| *Curvularia lunata* | 2 | 11 | 1 | 2 | - | - | - | 13 | - | 2 | 11 | - |
| *Derchslera* | 11 | 8 | 6 | 8 | 14 | 17 | 6 | 11 | 7 | 13 | 15 | 5 |
| *D. halodes* | 5 | - | 3 | 2 | 3 | 9 | - | - | 7 | 5 | 12 | 2 |
| *D. spicifera* | 6 | 8 | 3 | 6 | 11 | 8 | 6 | 11 | - | 8 | 3 | 3 |
| *Fusarium* | 8 | 22 | 22 | 17 | 7 | 25 | 32 | 3 | 19 | 30 | 18 | 24 |
| *F. moniliforme* | - | 22 | - | - | 6 | - | 21 | 3 | - | 20 | 18 | - |
| *F.oxysporum* | - | - | - | 6 | - | 13 | 6 | - | - | - | - | - |
| *F. solani* | 8 | - | 22 | 11 | 1 | 12 | 5 | - | 19 | 10 | - | 24 |
| *Humicola grisea* | 17 | 18 | 4 | 7 | 3 | 10 | 5 | 5 | 10 | 7 | 11 | - |
| *Myrothecium roseum* | - | - | 7 | 1 | 3 | 2 | - | - | - | - | - | - |
| *Paecilomyces variotii* | - | 7 | 2 | - | - | - | - | - | - | - | - | - |
| *Penicillium* | 9 | 9 | 6 | 13 | - | 5 | 10 | 2 | 2 | 5 | - | 3 |
| *P. chrysogenum* | 4 | 9 | 6 | 13 | - | 5 | 4 | 2 | 2 | 5 | - | 3 |
| *P. funiculosum* | 5 | - | - | - | - | - | 6 | - | - | - | - | - |
| *Phoma humicola* | 9 | 14 | 10 | 9 | 4 | - | 10 | 2 | - | 7 | 9 | - |
| *Scopulariopsis brevicaulis* | | - | 4 | - | - | - | - | 6 | - | - | 4 | - 3 |
| *Stachybotrys chartarum* | 16 | 31 | - | 20 | 16 | - | 4 | 8 | - | 13 | 28 | - |
| Sterile mycelia | 17 | 9 | 16 | 7 | 9 | 18 | - | - | - | 9 | 6 | 25 |
| *Torula herbarum* | - | 10 | - | 2 | - | - | - | - | - | - | 5 | - |
| *Trichothecium roseum* | - | - | - | - | - | - | - | - | - | 1 | - | 1 |
| *Trichoderma hamatum* | - | - | - | - | - | - | - | - | - | - | 7 | 5 |
| *Ulocadium atrum* | 22 | 49 | 28 | 23 | 46 | 52 | 24 | 40 | 27 | 17 | 70 | 68 |
| Total counts | 274 | 359 | 269 | 266 | 232 | 282 | 231 | 216 | 122 | 256 | 351 | 190 |
| No. of genera | 12 | 15 | 13 | 11 | 11 | 10 | 11 | 11 | 8 | 13 | 12 | 9 |
| No. of species | 20 | 18 | 18 | 18 | 16 | 14 | 19 | 13 | 9 | 19 | 17 | 11 |
| Total genera = 18 | 19 | 13 | 12 | 16 | | | | | | | | |
| Total species = 28 | 28 | 22 | 21 | 23 | | | | | | | | |

member were the common in the studied regions. Some species were encountered only on one media and prevalent on other one. Also, numerous species were found only from one region and not from the others and vis versa.. These results were harmony with those obtained by El-Gali and Abdullrahman[21] who studied the soil fungi in El-Beida, Libya and showed that the most abundant fungi were *Alternaria, Aspegillus, Fusarium* and *Penicillium* spp. The distribution of these fungi in such habitats which are in close association with human activities supports that contact with such habitats is a risk factor for the infections caused by these fungal species. Therefore careful contact with such habitats especially for immunocompromised individuals must be controlled to avoid funal infections. Sharma *et al.*[55] found that dominant soil colonizers from subtropical forest were *Cladosporium, Trichoderma* and *Penicillium.*

Also, the results indicated that the widest spectrum of genera and species being isolated on Cz, followed by cellulose and the narrowest on 10% NaCl media. It is worthmentioin that *Ulicladium* species were prevalent on Czapek's medium supplemented with10 %NaCl. This indicated that these members were able to tolerate high salte stress and called haloterant fungi. The same results were obtained by Moubasher *et al.*[6]**.** They could isolate 87 species related to 31 genera from 96 newly reclaimed soil samples from Wadi El-Natrun, Egypt on three isolation media.

In the current work, the widest spectrum of total counts being isolated on Czapek's - cellulose and the narrowest on 10% NaCl media, whereas Czapek's with 1% glucose medium was intermediate. *Aspergillus, Fusarium, Myrothecium, Stachybotrys, Penicillium* and *Emericella* were regularly the most dominant genera possessing the highest proportions of propagules on the three isolation media. Numerous species were found only on one medium and not on the others media (Table 2).Also, in the literature a great number of fungi recorded in the present investigation on 10% NaCl medium were also identified in different parts of the world on high salt concentrations from Purerto Rico[56], Dead Sea[57], Red sea shore[58], Salt marsh soil in Egypt[48], solar salterns[59], saline environments in Slovenia[60] and Mondovi estuary[61,62]. From the previous genera the most prevalent species were: *A. flavus,, A. fumigatus, A. ochraceus, A. terreus, P.chrysogenum, P. funiculosum, P. oxalicum, F. sambacinum, P. solani, A. alternata, A. chlamydospora, U. atrum* and *U. botrytis.* Some species were encountered from three cities such as: *A. carneus, A. ochraceus, A. terreus, Cladosporium cladosporioides, Myrothecium verrucaria, P. chrysogenum,* and *P. funiclosum.*, or two cities like: *A. alternata, A. brasiliensis, B. piluliferum, C. specifer, E. nidulans, Humicola fusco-atra, P. oxalicum, P. glomerata, Stachybotrys chartarum* and *U. tuberculatum* and not on the others. The remaining genera and species were isolated in rare frequency of occurrence found only in one city (Table 2). These species were also, isolated in high or rare frequency of occurrence from different habitats as reported by several researchers all over the world[4,53,63].

The isolated species have been increasingly reported recently to be the etiologic agents of serious diseases in human through traumatic inoculation[64-66]. There have been many reports of infection caused by black moulds in healthy individuals and in immunocompromised patients[67]. Also skin infections due to *Alternaria* in kidney transplant recipient have been reported by some investigators[68]. The prevalent clinical

**Table 4.** Flourescence (at 365 nm) of *Aspergillus flavus* strains isolated from the examined soil samples

| Isolates (No.) | Flourescence on plates | Source | Medium |
|---|---|---|---|
| *Aspergilus flavus* (1) | -ve | Rafha | Cz |
| *Aspergilus flavus* (2) | -ve | Rafha | Cz |
| *Aspergilus flavus* (3) | Aflatoxins B1, B2, G1, G2(500 µg/ L) | Linah | Cz |
| *Aspergilus flavus* (4) | B1 (50 µg/ L) | Al-Uwagilah | Cz |
| *Aspergilus flavus* (5) | B1, G1 (200 µg/ L) | Al-Uwagilah | Cz+NaCl |
| *Aspergilus flavus* (6) | -ve | Arar | Cz+NaCl |

features caused by *C. herbarum, C. cladosporioides*, and *C. sphaerospermum* included, infection of skin[65], corneal ulcer[69], brain abscesses[70], pulmonary ball[71] and dental granuloma[72]. Therefore careful contact with such habitats especially for immunocompromised individuals must be controlled to avoid funal infections.

**Fungi recovered from air**

Airborne microbial contamination is a significant issue in the crowded centers and cities, as air serves as a transmission vehicle for pathogens that have linked with adverse health effects ranging from infectious diseases to allergies[19].

Knowledge of species and density of outdoor airborne fungi in the studied environment can be especially important in the diagnosis and treatment of these diseases. In the present work the distribution of air fungal flora were performed during October – December 2015 over four governorates at the Northern border region, Saudi Arabia. Gravtational setting method using Pitri-dishes containing three isolation media wrer applied.

Exposed culture plates in various locations at the four cities as previously described produced a total of 28 species related to 18 genera on the three types of media used. Al-Falih[73] screened the air-borne fungal flora inhabiting school environments at different places in Riyadh city, Saudi Arabia. He could isolate 36 species belonging to 6 genera from the examined area. In Turkey, Topbas *et al.*[23] evaluated the prevalent species of airborne in the outdoor environment in Trabzon city, Turkey. Twelve genera of fungi were identified. It has been reported that the above genera should always be considered as a cause of fungal allergy[74]. In Iraq Muhsin and Adlan[28] made an assessment of air quality by examining outdoor airborne fungi among three sites over four seasons of the year 2009 in Basrah city (Iraq). They noticed that nine fungal genera including 16 species were prevalent in the air samples.

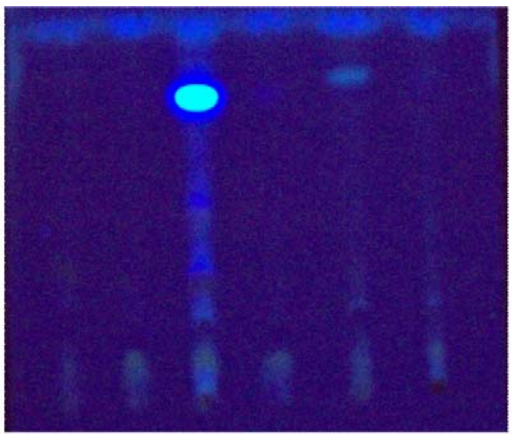

**Fig. 1.** Alfatoxin detected on thin layer chromotography (TLC) plates,Lane -1.,2 (negative); Lane 3 (mixture of flatoxins $B_1$, $B_2$, $G_1$, $G_2$), Lane 4 (aflatoxin $B_1$), Lane 5 (aflatoxin $B_1$ and $G_1$) and Lane 6 (negative)

As shown in Table (3) the highest level of reproduction in the terms of the number of colonies was observed in Rafha (274, 359, 269 colonies) followed by Arar (256, 351, 190 colonies), and the less was shown in Linah (266, 232, 282 colonies) and Al-Uwaygilah (231, 216, 122 colonies) on the three media, respectively. Also, when the the results analyzed with respect to the number of fungi found, Rafha ranked first with 28 species and 19 genera, and Arar occupied the second with 23 species and 16 genera. Regions Linah and Al-uwaygilah came the third and the fourth with 22 and 21 species and 13 and 12 genera, respectively. In most regions studied the high total counts and the widest spectrum of genera and species being isolated on Czapek's-glucose agar and the narrowest was encountered on Czapek's glucose with 10% NaCl, whereas Czapek's –cellulose media was intermediate. In the air, the dematiaceous fungi outnumbered the hyaline ones. Environmental factors such as atmospheric conditions, high light intensity, and deep diurnal fluctuations of temperature, wind and humidity may induce selective effects for advantage of the dematiaceous fungi over the hyaline like. Also, the melanin-containing fungi are more adapted to survive the injurious effects of these conditions. The present study showed similarities in both quantitative and qualitative compositions to many comparable investigations in tropical and subtropical areas such as Iraq, Iran, Laybia, Turkey, Kuwait, Egypt, as well as other studies in Saudi Arabia[6,64,75].

In the current study the most reproductive genera were *Alternaria*, *Cladosporium*, *Aspergillus*, *Ulocladium*, *Humicola* followed by *Derchslera*, *Fusarium*, *Stachybotrys* and *Penicillium*. From the above

genera *A. alternata, A. niger, A. fumigatus, A. terreus, C. cladosporioides, U. atrum, H. grisea, A. spicifera, F. monoliforme, S. chartarum* and *P. chrysogenum* were the most prevalent species. These results are comparable to those obtained from several studies in the world. These genera also exhibited a high level of reproduction allover the world. In Riyadh, Hasnain *et al*.[76] in a study performed in outdoor air In Riyadh, it was reported that the genera of *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Ulocladium* were the most common. Hasanain *et al*.[77] found that the genus *Ulocladium* emerged to be one of the five most prevalent fungi in the outdoor environment of three rgions (Riyad, Jeddah and Al-Khbar), Saudi Arabia. They observed that the percentage of *Ulocladium* in the air varied at all sites studied with highest composition being 7% of the total air spora. *Alternaria* is known to be a potent allergen world-wide and considered to be the third important allergen, after ragweed and grass pollen, as a natural cause of allergy in the United states[78]. Al-Suwaine *et al*.[75] studied the allergenic fungi and their seasonal fluctuation at two different sites (Al-Batha and Al-Ulia) in Riyadh city. They indicated that airborne fungi were grouped into major and minor components depending upon their frequency of appearance and catch percentage in the air. *Cladosporium, Penicillium, Aspergillus, Alternaria and Ulocladium* spp.were included as major components. While, minor components included *Drechslera, Rhizopus, Fusarium* and *Stachybotrys* spp. In a study performed in Ankara**,** Turkey, Mete *et al*.[79] reported that the most prevalent fungal genera were of *Rhizopus* (54%), *Cldosporium* (14.3%), *Penicillium* (12.4%), and *Alternaria* (4.7%). In a study conducted with 339 school children in Australia, Downs *et al.*[74] idicated that *Alternaria* allergens contributed to severe asthma in regions were exposure to the fungus was high. Also, In Turkey, Topbas *et al*.[23] evaluated the prevalent species of airborne in the outdoor environment in Trabzon city, Turkey and noticed that *Alternaria* (26.3%), *Cladosporium* (8.1%), *Penicillium* (26.8%) and *Fusarium* (13.2%) were the most prevalent fungal genera. In Iraq, Muhsin and Adlan[28] made an assessment of air quality by examining outdoor airborne fungi among three sites over four seasons of the year 2009 in Basrah city (Iraq). They noticed that the most predominant fungi belonged to the genera *Cldosporium*, *Penicillium*, *Alternaria* and *Aspergillus*. Also, highest counts of the fungal isolates were recorded for *C. cladosporioides* (31.3% frequency), followed by *P. notatum* (= *P. chrysogenum*, 11.9%), *A. alternata* (10.0%) and *A. niger* (5.8%). In Libya, El-gali and Abdullr[21] assessed the culturable airborne fungal colony and types in different seasons of 15 homes in different localities from April 2013 to March 2014 in Lybia. *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium solani*, *Penicillium chrysogenum* and *P. digitatum* were the predominant genera and the abundance of genera varied by season. Spores of the previous species are generally considered to be important causes of allergic rhinitis and asthma[80] .These genera have been reported among the predominant soil dematiaceus hyphomycetes in Eastern Tibet[81]. It may be attributed to size and nature of their conidia (small, dry and carried in long chains) which facilitate thir dispersal by air[82].

The remaining genera and species were isolated in low or rare frequency of occurrence. Also, some species were recovered from one city and not from the other and on one medium and not on the other (Table 3). These results are nearly similar to those obtained from different places all over the world[16, 83-86].

**Aflatoxins**

The fluorescence at 365 nm of 6 *Aspergillus flavus* strains recoverd in the present work indicated that 3 showed intense blue colour indicating aflatoxins B1, B2 production and fluoresced green colour indicating aflatoxin G1, G2 production. From the positive results 1 strain produced aflatoxins B1, B2, G1, G2 (500 µg/ L); one strain produced B1 (50 µg/ L)and one istrain produced B1 and G1 (200 µg/ L) which isolated from soil of Linah and Al-uwagilah on Cz and Cz+ Nacl media (Table 4 and Fig. 1). The remaining tested strains (3) showed negative results. In this respect**,** Riba *et al*.[87] screened 150 strains isolates that related to *A. flavus* (144 isolates) and *A. tamari* (6) for aflatoxin production and found only 45 isolates were aflatoxigenic. In the screening of Ezekiel *et al.*[88] on 90 isolates of *Aspergillus* section *Flavi* found that only 35.6 % of the isolates produced the characteristic fluorescence of aflatoxins under 365 nm UV. More recently, Ismail *et al*.[89] screened 43 fungal strains and noticed that

all *A. flavus* strains showed blue colour with different intensities indicating aflatoxin B production and one of them fluoresced greenish yellow colour indicating aflatoxin G production.

## CONCLUSION

In soil the hyaline fungi were predominant over the dark-coloured ones. It may be due to the soil is densely populated by microorganisms and competition for extence is very sever. On the other hand, fungi are relatively protected from the injurious effects of atmospheric conditions, high light intensity, and deep diurnal fluctuations of temperature and humidity. These conditions may selectively favour hyaline fungi over the dark-coloured (melanin-containing) fungi Chimatic factors influence the overall levels of airborne microorganisms causing allergic diseases. Hmide climates offer more opportunities for growth of varous groups of allergen producing organisms, especially fungi. Coastal areas have high humidty and horticultural practices throughout the year also provide sufficient moisture for fungal growth. This study provided some information regarding the air borne fungal composition at the Northern border region of Saudi Arabia and suggesting a further investigation to correlate between the common human allergies among the population and the incidence of airborne fungi in the environment of this cities. Knowledge on airborne fungi is important as it is considered a potential public health problem and data can be used as a base to develop criteria for assessing outdoor air quality in Saudi Arabia. Also, some of the isolated species especially related to *Aspergillus flavus* group are well-known as toxeginic and constitute a health hazard for human and animal.

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