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Isolation and Identification of Fungi from Cereal Grains in Libya

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Abstract: A total of 10 species of fungi belonging to 5 genera were isolated and identified from four cereal grains; wheat, barley, rice and maize collected from three Libyan cities known to grain producers (Al-Zawia, Subratah and Tripoli) on 1% dextrose-Czapkes agar medium at 28 ± 2°C for 7-15 days using seed-plate method. Two species of *Alternaria* (*A. raphani* and *A. tenusinae*); two species of *Aspergillus* (*A. flavus* and *A. niger*); three *Fusarium* (*F. graminearum*, *F. moniliforme* and *F. solani*); one *Rhizopus* species (*Rhizopus stolonifer*) and two species of *Penicillium* (*P. digitatum* and *P. notatum*) were isolated from the grains. The densities of these fungi and their frequencies of occurrence have been investigated. It can be concluded that for human public health, cereal grains of production chain must be subjected to quality control and microbiological examinations.

Keywords: Cereal Grains, Mycoflora, Seed-Plating Method

1. Introduction

High production of crops requires using high quality of seed. However, spore-forming fungi play an important role in deterioration of seed quality, which leads to high economic losses in crop yield [19]. Contamination of agricultural products, cereals and oil seeds in particular are the main sources of diseases in the human and animal food chains [17]. Cereal products, which provide income of important nutritional substance for human health. The quality and safety of agricultural product are of major concern due to increasing occurrence of chronic diseases associated with consumption of contamination [2].

One of the best methods to determine the natural and extent of the fungal colonization is the direct plating technique. This method is very useful for the quality evaluation of bulk grain. The genera of fungi of greatest importance in causing poisoning in humans and domesticated animal are *Aspergillus*, *Fusarium* and *Penicillium* [17, 19].

The fungi including *Aspergillus*, *Fusarium* and *Penicillium* genera are responsibole for majority of agricultural mycoflora contamination. These fungi are common components of the microbial flora associated with many agronomic crops, including corn and sorghum [13]. Wheat were also found to be contaminated in variable amounts by genera of fungi *Alternaria*, *Aspergillus* and *Fusarium* [18]. Microorganisms get on grain in different ways, most often with dust from soil, surface of plant remnants during harvesting, transportation, storage and processing [8, 19].

Numerous investigations have been carried out on cereals grains contamination all over the world [3, 17, 2, 9]. The grains fungal contamination have been carefully studied in several areas, and several grain types [11, 9].

In Libya, no published studies exist mycoflora contamination of cereal grains. Therefore, the purpose of this research was isolation and identification of fungi from four cereal grains (wheat, barley, rice and maize) which grown in three cities (Al-Zawia, Subratah and Tripoli) in Libya.

2. Materials and Methods

2.1. Collection of Samples

Four cereal grains (wheat, barley, rice and maize) were collected from Al-Zawia, Subratah and Tripoli in Libya. Each sample was put in sterile poly ethylene bag. Sealed to minimize the loss of water- content, transferred to the mycological laboratory and kept in a cool place (3-5°C) till fungal cultivation, isolation and identification.

2.2. Determination of Moisture Content of Cereal Grains

Twenty grams of each cereal grains samples were milled and dried in an oven at 105°C for 24h, then cooled in desiccator and re-weighted to constant weight. The moisture content of dry weight according to the technique of International Seed Testing Association [6].

2.3. Determination of Germinability of Cereal Grains

One hundred seeds of each sample were incubated at 25°C over a pad of moist sterile filter paper placed in sterile Petri dishes for 7 days. The grains with healthy roots and plumules were counted and the counts were expressed as percentages of the numbers of tested grains.

2.4. Determination of Cereal Grains -Borne Fungi

Seed plate method as described by [19] was used for isolation of fungi. Modified 1% dextrose, Czapkes agar medium (gL^{-1}, sodium nitrate 3.0, magnesium sulphate 0.01, dextrose 10.0, agar agar 15.0-20.0, pH 7.3 ± 0.1) was used as cultivation and isolation of medium. Chloramphenicol (0.5 mg mL^{-1}) and rosebengale (30ppm) were added to the medium as bacteriostatic agents. Ten plates were plated per sample, five for each method were used for each sample. Incubated plates were incubated at 28 ± 2°C for 7-15 days prior to visual differentiation and counting colonies. The colonies of slow growing fungi as well as mycelial bits were transferred to slants with special media to ensure precise counting, then to plate for identification.

2.5. Identification of Fungi

The following references were used for identification of fungi (based on purely morphologically macro-and microscopic characteristics). [16, 14, 15, 4, 12].

3. Results and Discussion

3.1. Determination of Moisture Content and Germinability of Cereal Grains

The moisture content of wheat, barley, rice and maize cereal grains samples (on oven dry basis) was relatively low and ranged between 6-8%, 6-8%, 5-11% and 5-8%, respectively. There is no significant difference between germinability of the two types of grains tested and germination rates ranged between 30-100%.

3.2. Determination of Cereal Grains-Borne Fungi and Identification

The percentages of total counts (% T.C) and frequencies of isolation (F%) of the isolated fungi summarized in Table 1. In general, a total of 10 species of fungi belonging to 5 genera were isolated and identified from four cereal grains; wheat, barley, rice and maize. Two species of *Alternaria* (*A. raphani* and *A. tenusinae*); two species of *Aspergillus* (*A. flavus* and *A. niger*); three *Fusarium* (*F. graminearum*, *F. moniliforme* and *F. solani*); one *Rhizopus* species (*Rhizopus stolonifer*) and two species of *Penicillium* (*P. digitatum* and *P. notatum*) were isolated.

In wheat grains *Aspergillus* was the most dominant fungus in three cities. The total count of *Aspergillus* were 47.2%, 46.6% and 57.9% in Al-Zawia, Subratah and Tripoli, respectively. It is frequencies of isolation were 80%, 90% and 90% in Al-Zawia, Subratah and Tripoli, respectively. *Rhizopus stolonifer* followed *Aspergillus* in domination of wheat grains in both Al-Zawia and Subratah with total count about (21.3%) and (28%) respectively, but *Alternaria* followed it in domination (32.2%) in Tripoli. *Penicillium* was less dominations and frequencies. (The statement is quiet confusing OK. Done) These results comes in accordance with that of [7] who found that out of 24 genera isolated from feeding wheat, *Aspergillus*, *Acremonium*, *Alternaria*, *Penicillium* and *Rhizopus* were the most frequently isolated. The results in this study are also in agreement with that of [17].

In case the barley grains, the most common fungus isolates was *Rhizopus stolonifer* in Al-Zawia and Subratah recording percentages of total count 47.0% and 46.6%, respectively. Whereas, *Aspergillus* was the most common (53.5%) and most frequent (100%) on barley grains collected from the three cities. In all cases *Alternaria* and *Penicillium* were less common on barley grains. *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* were found to be the dominant (are they dominant species found in barley? If so, then better use "dominant" than "predominant" that word will make confusion to the reader OK. Done) mycoflora on barley grains collected from several localities in the world [17, 1].

In case of rice grains, *Aspergillus* was the dominant genus in all set ups. Its percentages of total count were 46.0%, 69.3% and 53.1% and its frequencies of isolation were 80%, 100% and 100% for Al-Zawia, Subratah and Tripoli, respectively. *Fusarium* was the second to *Aspergillus* and frequency on rice grains collected from both Al-Zawia and Tripoli while it was not recorded at all rice grains collected from Subratah *Alternaria* was less common while *Penicillium* was rare in all cases. Mycoflora is a major cause of deterioration in rough rice and resulted to decrease its quality, economic value and perhaps in quantity. Some of the most common fungal genera found in the rice were *Biopolaris*, *Pyricularia*, *Fusarium*, *Alternaria* and *Epicoccum* [5]. It was found out that the common genera that caused the deterioration of stored rice were *Aspergillus* and *Penicillum.*

Penicillium was also high in maize grains. Its percentages of total count were 60.4%, 58.2% and 61.7% for Al-Zawia, Subratah and Tripoli, respectively. Followed by *Fusarium* and *Aspergillus* in percentage and frequency on maize grains in all set ups. The fungal population of freshly harvested corn grains collected from regions in Brazil were reported by [3]. Composed mainly of *Fusarium*, *Penicillium* and *Aspergillus* species. Among the genera of *Fusarium* and *Aspergillus*, the most frequently isolated species were *F. moniliforme* and *A. flavus*, respectively. Moreover, it has been reported that the

most serious maize contamination problems are due to *A. flavus* and *F. moniliforme* [17, 10].

Table 1. *Percentages and frequencies of fungi isolated from cereal grains from three cities in Libya.*

Cereal / Fungi	Maize		Rice		Barley		Wheat	
	%T.C	F%	%T.C	F%	%T.C	F%	%T.C	F%
Al-Zawia								
Alternaria	13.0	30	6.8	30	12.4	40	-	-
A. raphani	8.3	20	0.8	10	-	-	-	-
A. tenusinae	4.6	10	6.0	20	12.4	40	-	-
Aspergillus	47.3	80	17.9	90	64.1	80	14.3	70
A. flavus	13.0	70	9.7	80	41.6	80	9.9	60
A. niger	34.3	80	8.2	70	22.5	70	4.4	40
Fusarium	0.93	10	26.9	60	23.6	80	25.3	90
F. graminearum	-	-	6.0	10	23.6	-	-	-
F. moniliforme	0.93	10	10.5	20	-	80	25.3	90
F. solani	-	-	10.5	30	-	-	-	-
Rhizopus stolonifer	21.3	80	47.0	100	-	-	-	-
Penicillium	13.0	70	1.5	10	-	-	60.4	100
P. digitatum	0.93	10	-	-	-	-	-	-
P. notatum	12.01	70	1.5	10	-	-	60.4	100
Subratah								
Alternaria	16.1	40	6.1	30	15.0	90	-	-
A. raphani	-	-	-	-	-	-	-	-
A. tenusinae	16.1	40	6.1	30	15.0	90	-	-
Aspergillus	46.6	90	25	90	69.3	100	18.9	70
A. flavus	11.9	70	8.8	70	25.5	90	11.5	50
A. niger	34.7	90	16.2	80	43.8	90	7.4	30
Fusarium	2.5	20	15.5	30	-	-	23.0	10
F. graminearum	-	-	-	-	-	-	-	-
F. moniliforme	2.5	20	15.5	30	-	-	23.0	10
F. solani	-	-	-	-	-	-	-	-
Rhizopus stolonifer	28	80	46.6	100	6.5	10	-	-
Penicillium	6.8	60	1.4	10	9.2	70	58.2	90
P. digitatum	-	-	-	-	-	-	-	-
P. notatum	6.8	60	1.4	10	9.2	70	58.2	90
Tripoli								
Alternaria	32.2	80	0.7	10	17.5	90	-	-
A. raphani	21.7	60	0.7	10	10.2	60	-	-
A. tenusinae	10.5	40	-	-	7.3	40	-	-
Aspergillus	57.9	90	53.5	100	53.1	100	13.8	60
A. flavus	57.9	90	40.1	90	47.5	100	8.5	50
A. niger	-	-	13.4	30	6.6	10	5.3	40
Fusarium	7.2	30	45.8	90	28.8	90	24.5	90
F. graminearum	-	-	-	-	-	-	-	-
F. moniliforme	7.2	30	45.8	90	26.6	90	24.5	90
F. solani	-	-	-	-	2.2	10	-	
Rhizopus stolonifer	-	-	-	-	-	-	-	-
Penicillium	2.6	10	-	-	0.6	10	61.7	100
P. digitatum	-	-	-	-	-	-	-	-
P. notatum	2.6	10	-	-	0.6	10	61.7	100

%T.C: Percentage of total count of fungal isolates. $\%T.C = \frac{\text{no.of isolates}}{\text{total no.of fungal isolates}} \times 100$

F%: Percentage frequency of occurrence. $F\% = \frac{\text{no.of times of isolates}}{\text{no.of samples}} \times 100$

(-): No fungal species detected.

4. Conclusion

In conclusion, cereal grains are main important food of people in most regions of the world. The mycoflora diversity on freshly harvested Libyan cereal grains is relatively low, only 10 species of fungi belonging to 5 genera were isolated. So, for human public health, cereal grains of production chain must be subjected to quality control and microbiological examinations.

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