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Natural occurrence of fungi in stored wheat grains in Egypt

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Abstract: Moulds can destroy wheat and its products both before and after harvest, when they are being produced and stored. Thirty samples of stored wheat grains were taken from various markets in Mansoura city, Dakahilia Governorate, Egypt and analyzed for the presence of naturally occurring moulds. The examined samples had a high prevalence of several fungal populations, with counts ranging from 16 to 34 colonies/g. Nine genera and twenty-two species have been distinguished using the conventional agar plate method. *Alternaria*, *Aspergillus*, *Botryotrichum*, *Cladsporium*, *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma*, and *Ulocladium* are the genera that were identified during this study. The most common fungal genus observed was *Aspergillus* (100%) and was followed by *Penicillium* (83.3%). *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Alternaria alternate*, and *Penicillium chrysogenum* were the most prevalent fungal species. These observations showed that more firm actions need to be applied by farmers and food industries during pre-and post-harvest practices to avert or reduce mold contamination and hence mycotoxins production in wheat grains.

keywords: Wheat grains, Contamination, Fungi, Storage, *Aspergillus flavus*

1. Introduction

The condition of the ecosystems that produce food determines the health of human populations primarily, therefore supply pressure can have an impact on both the amount and quality of food that is available. Individuals who reside in rural and underdeveloped nations and who typically rely on food produced locally encounter problems restrictively associated with food security, safety and quality which are the key problems that are affecting the entire world population. Before, during, and after harvest, the majority of food and agricultural products may come into contact with a wide range of microbiological pollutants **[1]**. Moulds are one of the biggest threats to cereal grain deterioration in storage and cereal food that is generated from it **[2]**. Due to their versatile nutritional requirements, they have a worldwide distribution and are common contaminants of several food and feedstuffs and with suitable conditions of temperature and humidity, proliferate on various beverages and commodities **[3]**. Moulds had been categorized as grade second after insect pests as a reason for spoilage during the storage of seeds **[4]**. Contamination by fungi causes large quantities of food to be wasted every year **[5]**. Annual foodstuff loss of 16% is due to microbial diseases out of which fungi alone have contributed about 80% **[6]**. Fungal invasion of the majority of agricultural supplies can occur before to, during, or following harvesting, as a result of inappropriate handling, drying, improper transportation, and poor storage (Various types of shelters utilized repeatedly for storage are typically composed of mud, uncovered shads, fabric or polyethylene bags, and ceramic containers with varying dimensions, inadequate hygiene of producers and congenital climatic conditions, retailed in market openly in unhygienic conditions **[7]**. From the agricultural commodities, cereals and its products represent an important group since they are the main food for humans throughout the world. Cereal contamination is not a problem of one special geographic place or any climate region but it is globally. In terms of planted area, wheat is among the most essential and strategically placed cereals worldwide, with corn, barley, and sorghum following in significance. More than a billion people on the planet rely on wheat grains for their diet than any other type of plant or animal **[8]**. After rice, wheat is the cereal crop that is produced worldwide the most. Furthermore, wheat has a higher protein and calorie content than virtually every other food agricultural product, accounting for more than 20% of daily caloric consumption **[9]**. The primary reason why storage is important in agriculture is because, although agricultural output is seasonal, the needs for crop products are consistent throughout the year. The following conditions promote the growth of fungi during storage: (1) grain nutritional composition; (2) temperature and moisture levels (3) biotic factors, such as the existence of insects in products that are stored or competition. Because stored product insects create moisture and distribute fungal spores throughout the commodity, lots invaded by these insects have higher concentrations of storage fungi. **[10]**. Fungal action in the grains can destroy lipids and proteins or change their digestibility, produce volatile byproducts causing unpleasant odors, cause losses in the germination process baking, and grinding quality, participate in warming and revenue losses in dry material via the uptake of carbohydrates as source of energy, and affect its apply as feed for animals or as seeds. Every year, diseases cause the loss of roughly 20% of the wheat that would otherwise be readily accessible for consumption and feed **[11]**. The primary reason for quality degradation and spoiling is the activity of pathogenic microorganisms, which interact with the grain, the storage facilities' surroundings, and each other. The major reasons for the effects that fungus may have on the quality of grain, including germinability, are the post-harvest handling of the major grains and the prevailing environmental conditions. This study's primary objective was to quantify the mycoflora related to stored wheat grains to gather information on the likelihood of a health risk to those who consume these grains in order to avoid or manage the disease.

2. Materials and methods

Collection of samples:

Thirty samples of wheat grains were haphazardly gathered in clean, labeled, sterilized bags from several local marketplaces in Mansoura city, Dakahlia governorate, Egypt between December 2021 and April 2022. These samples appeared to be in excellent condition and came from those that were sold for human use. Wheat grains were sampled using the methods supplied by the European Commission and the International Organization for Standardization **[12]**. Each aggregate sample is 1kg in total weight. For each sample, 3 replicates were taken and mixed to prepare one combined sample. Each sample was positioned in a sealed sterile polyethylene bag. After being collected, the samples were immediately transported to the laboratory and surveyed for the presence of fungi and the rest of the samples were kept frozen at 5^{o}C for further analysis **[13]**.

Isolation and purification of mycoflora associated with the collected samples:

The method of direct agar plate was used to determine the total number of fungal colonies **[14]**. Using sterile forceps, about 0.5 gram (10 grains per plate) of each sample were haphazardly inoculated into a Petri dish that contained a sterilized potato dextrose agar medium (PDA) (200 g potato, 20 g dextrose, 20 g agar and 1 L distilled water) supplemented with 0.5 mg chloramphenicol/L to defeat the growth of bacteria. After preparing three replicates, the plates were incubated for 5-7 days at 28±2°C. Following incubation, the developing fungal colonies were isolated, and each isolate's purity was evaluated by streaking it over Czapeck-Dox agar medium (30 g sucrose, 3 g sodium nitrate, 0.5 g potassium chloride, 0.5 g magnesium sulfate, 0.01 g ferrous sulfate, 1 g dibasic potassium phosphate, 20 g agar and 1 L distilled water) after which it was transferred to PDA (potato dextrose agar medium) slants and stored at 4°C until identification. For each sample, the number of fungal colonies was counted and calculated in gram. To choose species, that belong to the genera *Aspergillus* and *Penicillium* and to reduce the spreading of fast-growing fungi (almost phycomycetes), a sterile solution of 8% NaCl was added to the medium as sodium chloride is typically applied to regulate the water activity **[15]**. The percentage of total counts (TC) and isolation frequency (FR) of species within a genus of fungi were calculated as follows **[16]**:

$$TC\ (\%) = \frac{Number\ of\ isolates\ of\ a\ species\ or\ genus}{Total\ number\ of\ fungal\ isolates} \times 100$$

$$FR\ (\%) = \frac{Number\ of\ samples\ with\ a\ species\ or\ genus}{Total\ number\ of\ samples} \times 100$$

Identification of fungal isolates:

Following their subculturing on the Czapeck Dox agar medium, the resultant fungal colonies were recognized by morphological and microscopic features, sporulation, and colony color. Each pure culture was then described and identified. The examination was done as described by **[17]** using needle mount preparation. The following widely recognized keys for the identification of various isolated fungi were used to assist in the identification of fungal genera and species **[18-27]**.

3. Results and Discussion

The fungal communities isolated from the wheat grain samples are displayed in Table (1). Twenty-two species belonging to nine genera were identified throughout this investigation namely: *Alternaria*, *Aspergillus*, *Botryotrichum*, *Cladsporium*, *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma* and *Ulocladium*. *Penicillium*, *Fusarium*, *Alternaria*, *Mucor* and especially *Aspergillus* (belonging to section *Flavi* and *Nigri*) were the most vital fungal species, and are frequently isolated from wheat grains in Algeria **[28]**. Comparable observations were almost stated by **[29-34]**.

reported that natural extrinsic contamination by dust after storage in humid circumstances was the cause of the fungal species contamination of food products There are two ecological groups of fungi: storage fungi and field fungi. Field fungi have been found to attack immature or developing seeds while it is still attached to the plant, *Alternaria*, *Helminthosporium*, *Fusarium*, and *Cladosporium* are the main field fungal genera. On the other hand, storage molds are found on plants in damp environments and are typically associated with things that are kept. These fungi are mostly *Aspergillus* and *Penicillium* species. Sometimes it is difficult to distinguish clearly between "field fungi" and "storage fungi," since fungal growth can occur both in the field and during storage **[25]**.

Furthermore, the results obtained in Table (1&2) showed also that, of all the genera identified, *Aspergillus* was the most common genus to be isolated, appearing in nearly all samples (100%) and accounting for 46.2% of the total fungal count. In this connection, Soliman **(16)** analyzed five different cereal grain varieties: sorghum, rice, wheat, maize and barley. The samples were taken from three known grain-producing areas in Egypt: Kafer el-Sheikh, Dakahilia, and Gharbia, the results revealed that the most common and often occurring fungus in relation to wheat grains throughout the three departments was *Aspergilllus*. In this regard, *Aspergillus's* overall count percentages were 47.2, 46.6, and 57.9% in Dakahlia, Gharbia, and Kafer el-Sheikh, respectively, although its isolation frequencies were 80%, 90%, and 90% in each of those same locations **[16]**. The frequency at which fungus occur in wheat grain samples collected from several Egyptian governorates was studied by **[15]** who showed that *Aspergillus* was the most common fungal genus, accounting for 59.67% of the total fungal count. *Penicillium* and *Fusarium* spp. were next in order. *Aspergillus* species produce a variety of enzymes which represent the initial steps in gaining entrance to nutrients from plants and food commodities **[35]**.

In this investigation, *A. aflatoxiformans*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraecous*, *A. oryzae*, *A. parasiticus*, *A. tamarii*, *A. terreus*, and *A. versicolor* were the ten *Aspergillus* species that were found. The most frequently occurring and widely distributed species found in nearly all samples were *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus versicolor*. These species accounted for 100%, 96.6%, and 40% of their frequency of occurrence, providing 18.1%, 7.2%, and 4.3% of the overall count of fungi, respectively. In this regard, **[36]** designated that *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger*, *Aspergillus ochraceus*, and *Aspergillus versicolor* were the most frequently isolated fungi from a variety of wheat grain samples **[36]**. **[31]** stated that amongst the diverse species recognized from wheat grains at various locations and storage times, *Aspergillus flavus* and *Alternaria triticina* were the most common species, but their frequencies varied **[37]**. The most common indigenous *Aspergillus* species that contaminated grains was *Aspergillus flavus*. According to observations from different countries, *A. flavus* is the most common species of *Aspergillus* contaminating wheat grains and their products, and the genus *Aspergillus* is the most common contaminant of stored wheat and flour **[28, 38-41]**. In addition, in warmer climates, *A. niger* is common in stored foods as well as field crops. The remaining species were all isolated and very occasionally occurred. *Aspergilli* are more common than other molds, which may be explained by their saprophytic nature and capacity to colonize a wide range of substrates due to the secretion of several hydrolytic enzymes **[42]**.

As demonstrated in Table (1&2), the second most dominant genus was *Penicillium*, which was found in 83.3% of the samples examined, accounting for 14.6 % of all the fungal count. *Penicillium chrysogenum*, *P. digitatum*, and *P. notatum* were the three species that were recognized; of these, *P. chrysogenum* was the most frequently found, accounting for 60% of all samples and 8.1% of the total number of fungi. A study by **[23]** indicated that fungi that are most prevalent and have been isolated from combined harvester wheat on glucose Czapek's Dox agar at 28 °C were *Aspergillus flavus*, *A. ochraceus*, *A. niger*, *A. terreus*, *A. fumigatus*, *Penicillium citrinum* and *P. chrysogenum* **[29]**. *Aspergillus sp.* and *Penicillium sp.* were amongst the storage fungi that were prevalent in Brazilian wheat **[43]**. **Roige *et al*. [44]** isolated ten genera of fungi from wheat grain samples in Argentina. By far the most widespread genus (42%) was *Penicillium*. Of the samples that tested positive for *Penicillium*, 29% had 106 cfu/g of feed **[44]**. Nearly identical outcomes were previously published by **[34, 36]**.

Rhizopus, on the other hand, was ranked third after *Aspergillus* and *Penicilium* and was found rather frequently, accounting for 70% of all samples examined and 10.7% of all the fungal count. It was represented by a single species namely: *Rhizopus stolonifer*. These results are in agreement with **[16]** who indicated that in terms of the dominance of wheat grains in Daqahlia (21.3%) and Gharbia (28%) *Rhizopus stolonifer* came after *Aspergillus* **[16]**. **[34]** stated that *Alternaria tenuis* and *Alternaria alternate* were the most fungal species frequently occurred and isolated from almost all tested wheat grain samples in all diverse Governorates followed by *Rhizopus stolonifer* comprising 115 isolates (25.0 %) **[34]**. In contrast, **[45]** isolated *Rhizopus stolonifer* in low frequency from wheat grain samples encountering 30% of all tested samples **[45]**.

As shown in Table (1&2), moderately frequent fungal genera *Alternaria* and *Fusarium* accounted for 66.7% of all samples examined and 9.7% and 6.6% of all the fungal count, respectively. Three species namely: *Fusarium moniliform*e, *F. oxysporum*, and *F. solani* represented the genus *Fusarium*, while only one species called *Alternaria alternate* represented the genus *Alternaria*. Our findings supported the observations of another research in Argentina which introduced the most prevalent fungal species of *Alternaria* and *Fusarium* as endogenous mycoflora **[46]**. revealed that the most common genera found in wheat were *Penicillium* (42%), *Fusarium* (27%), and *Alternaria* (25%) **[44]**. According to**. (45)** , *Aspergillus niger* and *Alternaria spp.* were the most frequent fungi found in the wheat grains, and *Fusarium spp.* was the third most common fungus detected there **[33]**. In another study in Egypt, *Aspergillus flavus* (86.66%), *Penicillium spp.* (73.33%), and *A. niger* (53.33%) were the most frequently isolated species from wheat grains. These were followed by *A. parasiticus* (33.33%), *Fusarium spp.* (33.33%), and *Alternaria* (46.66%) **[47]**. Pelhate (45) indicated that *Fusarium* was detected during harvesting owing to field contamination and it died as soon as the oxygen content dropped **[48]**.

Furthermore, a moderate frequency of *Cladsporium spp.* and *Ulocladium alternaria* was observed, accounting for 60% and 53.3% of the samples, respectively, giving 4.3% and 3.8% of the overall count of fungi. From wheat grains, several researchers isolated *Cladsporium spp.* **[32, 37, 45, 49, 50]**. Also, the isolation of *Ulocladium alternaria* has been reported by many researchers from wheat grains **[32, 51, 52]**.

The residual species were isolated with rare frequency of occurrence. Apart from *Aspergillus* and *Penicillium* species, these fungi were regarded as field fungi; nevertheless, it was discovered that these fungi experienced a discernible decrease during storage, and that storage fungi may take their place **[53]**.

It is imaginable that spoilage molds are a direct result of an open storage conditions, especially at the place of origin **[54]**. Contamination by fungi is affected by the environment, inappropriate handling, and inconvenient drying and storage of grains during cultivation and harvesting. Also, it was reported that fungal counts differ according to the year of production, the county, the crop and storage conditions prior to drying. Depending on the type of agricultural commodities and the fungus, numerous fungi were isolated with varying occurrences and percentages. Extending the storage duration resulted in a considerable rise in the overall fungal population of grains **[55]**. In the stored grain bulk, fungal development increases respiration, which releases heat and moisture into the surrounding air. Since Egypt's climate is characterized by high temperatures and relative humidity, *Aspergillus* species and other fungi can grow on grains. Besides, contamination occurs when grain is being stored and handled by handling and storage equipment that harbors minute amounts of spores from the harvest, or when spores already residing in storage structures cause contamination. Additionally, the farmers have employed antiquated and conventional agricultural methods that can enhance fungal infections **[47]**.

4. Conclusion

Concerning the regulation of the agriculture and food sectors, the security of food and feed for consumption by humans and animals ought to be the first priority. This study revealed that wheat grain samples were contaminated with either one or more molds with a higher prevalence of *Aspergillus* than other genera, with *Penicillium* coming in second. The presence of these fungi in wheat grains is a sign of possible health threats as some species of *Aspergillus* are known to cause food poisoning because of the production of toxins. Establishing a continuous control and monitoring program from grain production to consumption is crucial to decreasing the danger of fungal contamination and the subsequent production of mycotoxins. However, advanced training on this issue should be provided especially for farmers and agriculturists. By following the best standards for harvesting, managing, and storing food and choosing the right time to harvest it, this issue can be reduced or completely eliminated, posing no hazard to human health and minimizing the possibility of enormous financial loss.

List of fungal species recorded in this investigation

Alternaria alternate (Fr.) Keissler

Aspergillus aflatoxiformans Frisvad, Ezekiel, Samson & Houbraken

A. flavus Link

A. fumigatus Fresenius

A. niger V. Tiegh.

A. ochraecous Wilhelm = ***A. alutaceus*** Berkeley & Curtis

A. oryzae (Ahlburg) Cohn

A. parasiticus Speare

A. tamarii Kita

A. terreus Thom

A. versicolor (Vuill. Tiraboschi)

Botryotrichum piluliferum Sacc. & March

Cladsporium spp Link

Fusarium moniliforme Shel

F. oxysporum Schlecht

F. solani (Mart.) SACCARDO

Penicillium chrysogenum Thom

P. digitatum (Pers.) Sacc

P. notatum Westling

R. stolonifer (Ehrenb ex Fr.) Linder

Trichoderma harzianum Rifai

Ulocladium alternariae (Cooke) Simmons

Table (1) Total counts (colonies/g), isolation cases, occurrence notes for fungal genera and species, and percentage frequency of occurrence recorded in this investigation

Fungal genera and species	Total count	NCI and OR	% frequency of occurrence
Alternaria	72	20M	66.7
A. alternata	72	20M	66.7
Aspergillus	342	30H	100
A. aflatoxiformans	7	5R	16.7
A. flavus	53	29H	96.6
A. fumigatus	35	11R	36.7
A. niger	134	30H	100
A. ochraecous	23	7R	23.3
A. oryzae	15	6R	20
A. parasiticus	11	9R	30
A. tamarii	9	3R	10
A. terreus	24	6R	20
A. versicolor	32	12M	40
Botryotrichum piluliferum	10	6R	20
Cladosporium spp	32	18M	60
Fusarium	49	20M	66.7
F.moniliforme	14	8R	26.7
F. oxysporum	17	8R	26.7
F. solani	18	10R	33.3
Penicillium	108	25H	83.3
P. chrysogenum	60	18M	60
P. digitatum	21	8R	26.7
P. notatum	27	7R	23.3
Rhizopus	79	21M	70
R. stolonifer	79	21M	70
Trichoderma harzianum	19	5R	16.7
Ulocladium alternaria	28	16M	53.3

Table (2) Total count (colonies/g) of fungi by agar plate method at 28±2°C isolated from wheat grain samples

Number of samples / Genera & species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	% TC
Alternaria	−	4	5	5	3	3	3	9	3	8	3	2	−	−	3	−	−	−	2	5	2	3	−	−	3	4	1	−	1	−	9.7
A. alternata	−	4	5	5	3	3	3	9	3	8	3	2	−	−	3	−	−	−	2	5	2	3	−	−	3	4	1	−	1	−	9.7
Aspergillus	19	7	12	11	12	8	9	10	13	12	10	9	5	12	11	8	13	14	7	9	9	10	14	11	13	12	14	16	15	17	46.2
A. aflatoxiformans	−	−	−	−	1	−	1	−	−	−	−	−	−	−	−	−	1	1	−	−	−	−	−	−	−	−	−	−	2	−	0.94
A. flavus	5	2	1	1	−	1	1	1	1	1	1	2	1	2	2	3	2	2	1	4	2	1	1	2	2	2	3	2	1	3	7.2
A. fumigatus	4	1	4	−	5	−	4	−	4	−	−	−	−	−	1	−	−	−	−	−	−	−	−	−	5	−	2	−	2	3	4.7
A.niger	5	3	4	5	5	3	3	6	5	8	5	5	3	5	7	5	5	5	2	5	3	5	5	1	5	1	6	4	5	5	18.1
A.ochraecous	−	1	−	5	−	4	−	−	2	−	−	−	−	−	−	−	5	3	−	−	−	−	−	3	−	−	−	−	−	−	3.1
A. oryzae	−	−	−	−	−	−	−	2	−	3	−	−	−	−	−	−	−	−	2	−	2	−	−	−	−	−	−	4	2	−	2.1
A. parasiticus	−	−	−	−	−	−	−	1	−	−	−	2	1	−	1	−	−	−	1	−	−	−	−	−	1	2	−	2	−	−	1.5
A. tamarii	−	−	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	3	−	−	−	−	−	3	−	−	−	−	−	−	1.2
A. terreus	5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	5	−	−	4	3	4	−	3	3.2
A. versicolor	−	−	−	−	1	−	−	−	1	−	4	−	−	5	−	−	−	−	1	−	2	4	3	2	−	3	−	−	3	3	4.3
Botryotrichum piluliferum	−	−	−	−	−	−	−	−	−	−	−	−	−	−	3	−	1	1	−	1	−	−	−	−	−	−	−	2	2	−	1.4
Cladsporium spp	−	−	−	1	2	−	2	1	−	1	−	1	2	2	−	3	−	3	1	1	1	−	1	2	−	3	2	3	−	−	4.3
Fusarium	2	3	2	5	1	1	−	−	3	−	3	1	1	3	1	−	−	4	2	−	−	3	3	−	−	3	3	2	−	3	6.6
F.moniliform	2	−	−	−	−	1	−	−	−	−	1	−	−	2	−	−	−	2	−	−	−	1	−	−	−	−	3	2	−	−	1.9
F. oxysporum	−	−	−	2	1	−	−	−	1	−	−	−	−	−	−	−	−	2	2	−	−	−	3	−	−	3	−	−	−	3	2.3
F. solani	−	3	2	3	−	−	−	−	2	−	2	1	1	1	1	−	−	−	−	−	−	2	−	−	−	−	−	−	−	−	2.4
Penicillium	4	10	5	−	3	−	−	2	1	4	3	2	−	−	2	4	6	5	3	4	4	4	5	3	4	5	5	6	9	5	14.6
P.chrysogenum	2	5	5	−	3	−	−	2	1	−	3	−	−	−	2	−	−	5	3	−	2	2	5	−	2	5	3	−	5	5	8.1
P.digitatum	2	5	−	−	−	−	−	−	−	−	−	2	−	−	−	−	−	−	−	−	2	2	−	3	−	−	2	3	−	−	2.8
P.notatum	−	−	−	−	−	−	−	−	−	4	−	−	−	−	−	4	6	−	−	4	−	−	−	−	2	−	−	3	4	−	3.6
Rhizopus	7	6	7	6	2	5	4	2	−	−	2	3	5	3	4	3	5	−	−	−	−	−	−	2	4	−	2	2	3	2	10.7
R.stolonifer	7	6	7	6	2	5	4	2	−	−	2	3	5	3	4	3	5	−	−	−	−	−	−	2	4	−	2	2	3	2	10.7
Trichoderma harzianum	−	−	−	−	−	−	−	−	−	−	−	−	5	−	−	−	−	−	−	−	3	4	−	−	−	−	2	−	−	5	2.6
Ulocladium alternaria	−	−	1	1	−	−	1	1	−	1	−	−	−	3	−	2	−	3	1	−	1	−	3	2	−	2	2	3	1	−	3.8
Total counts	32	30	32	29	23	17	19	25	20	26	21	18	18	23	24	20	25	30	16	20	20	24	26	21	24	29	31	34	31	32	740
Number of genera	4	5	5	6	6	4	5	6	4	5	5	6	5	5	6	5	4	6	6	5	6	5	5	5	4	6	8	7	6	5	9
Number of species	8	9	8	9	9	6	8	9	9	7	8	8	7	8	9	6	7	11	10	6	10	9	8	9	8	10	12	12	12	9	22

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