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Mycological Quality Assessment of Powdered Groundnut Cake Sold in Selected Areas in Dutsin-Ma, Katsina State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to evaluate the mycological quality of powdered groundnut cake available in specified regions of Dutsin-Ma, Katsina State, Nigeria. A total of (40) samples were collected from various vendors in the selected areas. Standard laboratory methods were employed to assess fungal contamination, including culture-based techniques and microscopic examination.

The results revealed varying degrees of fungal presence across the sampled powdered groundnut cake. The predominant fungal species identified included (A. flavus, A. fumigatus, A. Lentulus, A. nidulans and A. niger, species and Rhizopus), indicating potential risks associated with consumption. Moreover, the study highlighted potential factors contributing to fungal contamination, such as improper storage, processing, and handling practices.

In conclusion, the mycological assessment underscores the need for enhanced quality control measures in the production, storage, and distribution of powdered groundnut cake within these regions. Implementation of proper hygiene practices, improved storage facilities,

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and education on food safety are recommended to mitigate fungal contamination and ensure the provision of safe and wholesome powdered groundnut cake to consumers in Dutsin-Ma, Katsina State.

Keywords: Mycological; groundnut cake; quality; assessments.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a mycotoxin-contaminated food that includes a variety of essential nutrients and is mostly produced in China, India, Senegal, Niger, Nigeria, the United States, and Sudan [1]. Groundnut seeds are low in trans fats and salt, and high in oil, protein, magnesium, manganese, niacin, lysine, fiber, phosphorus, and B vitamins [2]. They are made up of protein, amino acids, and fatty acids. As a result of the availability of these multiple important nutrients, which also attract mycotoxigenic fungus, groundnuts are understandably susceptible to mycotoxin contamination. Furthermore, groundnut accounts for around 21% of rural cash earnings for groundnut producers in Nigeria and approximately 70% of rural employment in Senegal. Naraian et al. [1]. Therefore, it is a significant source of protein, oil, cash and compound feed in many West African countries including Nigeria. Adams and Moss [3].

Groundnut cake, also known as kulikuli, is a popular snack made from groundnuts (peanuts) in Nigeria and many other parts of West Africa. Groundnut cake is a fried waste obtained after oil extraction from groundnuts that originated in northern Nigerian states such as Kano, Sokoto, Kaduna, Zamfara, Abuja, and Katsina [4]. Peanuts are roasted and crushed into a paste called "Labu" to make kuli-kuli. The paste is then blended with spices, salt, and occasionally ground pepper and sugar. Water is used to remove excess oil from the paste before molding it into the desired shape (chalk, donut, round balls, punched, cuboid, flat, wave, freeform, etc.). The addition of potash to the oil raises the boiling point of the oil. As a result of the oils being extracted from the kuli-kuli, more liquid oil is produced than before the process began. The formed peanut paste begins to firm and harden as it is heated and fried. After then, it is taken from the oil and allowed to cool [5]. Groundnut cake powder is made by crushing groundnut cake into a fine powder that can be used in soup recipes. Mengistu and Tolera. [6]. Groundnut cake is not considered a major food worthy of scholarly attention, but because it is hawked and sold in large quantities in various markets across Nigeria, there is an urgent need to evaluate its quality and microbial value on a regular basis, as failure to do so may result in mass food poisoning harmful to human health. However, groundnuts and their derivative foods are frequently susceptible to fungal degradation and mycotoxin (fungal toxin) contamination [7]. This could be due to the multiple nutrients it provides, or it could be due to unsanitary handling and storage time. Aflatoxins, Fumonisin, Deoxynivalenol (DON), Ochratoxins, Patulins, Coumarins, Zearalenols, Trichothecenes, Fusaric Acids, Fumagillin, and other fungal toxins are examples of mycotoxins. However, Aflatoxins (AFs) are the most frequent and widely used in Nigeria. Toxins are generated as extracellular metabolites by some fungus, such as Aspergillus spp. Abdul-lateef [5].

2. MATERIALS AND METHODS

2.1 Sampling Site

The samples were collected from the groundnut cake vendors at the following locations based on coordinate of latitude and longitudes; FUDMA take off campus (12°28.3080'N, 7°29.2990'E), Wednesday Market (12°27.6440'N, 7°29.2180'E), Gangare Mosque (12°27.3530'N, 7°30.0690'E) and GRA Mosque (12°27.5960'N, 7°30.5820'E) Dutsin-Ma Local Government, Katsina State, Nigeria.

2.2 Sample Size

Ten samples were gathered from each of the four above-mentioned locations, for a total of 40 samples.

2.3 Sample Collection

The samples were gathered because the product was sold in a sterile polythene bag. These samples were obtained directly from the supplier and promptly transported to the microbiological laboratory for testing. Bukar et al. [8].

2.4 Media Preparation

Potato dextrose agar was made according to the manufacturer's instructions (39g PDA dissolved in 1000 ml distilled water). The media was

autoclaved for 15 minutes at 15 psi and allowed to cool. To limit bacterial growth, chloramphenicol was added to the medium. The medium was then aseptically poured into each Petri plate and allowed to harden, as demonstrated by Adjou et al. [9].

2.5 Sample Preparation

The protocol given by Salau et al. [10]. In 9 ml of sterile distilled water, 1g of the sample was diluted. The mixture was shaken to homogenize it in a sterile test tube that represented the stock solution with a dilution of 10 and was labeled as 10^{-1}. Using a syringe, precisely 1 ml of the homogenate sample was transferred into the second sterile test tube containing 9 ml of sterile distilled water and labeled 10^{-2}. This procedure was performed again to obtain 10^{-3} and 10^{-4} dilutions, respectively, as described by John et al. [11].

2.6 Enumeration of Fungi

An aliquot of the serial diluents, typically 0.1 ml, was aseptically selected and spread onto the PDA medium with a sterile swab stick.

The plates were incubated at room temperature (25°C) for 4-7 days. The plates were checked for growth every four to seven days. Growth was detected in practically all of the plates following the aforementioned period.

The various species that appeared were morphologically observed based on their colony features, type of growth, or texture.

A colony counting chamber was used to do the fungal colony count, and the results were recorded as colony-forming units per gram (cfu/g). As demonstrated by Hell et al. [12].

2.7 Cotton Blue Stain and Identification of Fungi

A drop of lactophenol cotton blue was placed on a clean glass slide, followed by a small amount of actively growing fungi, and covered with a coverslip. Under x10 and x40 objective lenses, the glass slide was examined for microscopic features such as asci, ascospores, conidial, phialides, vesicles, and sporangiophores, among others. The fungal isolates were identified and characterized by comparing their colony shape and microscopic appearance to those of known taxa described by Kidd et al. [13].

2.8 Statistical Analysis

Microsoft Office Excel 2016 was used for the data analysis. The fungi isolated were recorded as frequency and prevalence. Two-Factor Without Replication Analysis of Variance (ANOVA) was used to compute and arrived at statistical decision and $p < 0.05$.

3. RESULTS

Table 1: displays the macroscopic (cultural) and microscopic (appearance of sporangia, spores, and hyphae) characteristics of the identified fungi recovered from powdered groundnut cake samples.

Table 2: shows that the GRA Mosque had the highest mean value of fungal load of powdered groundnut cake samples, with 6.61 x 10^5 CFU/g, while FUDMA Takeoff Campus and Gangare Mosque had moderate mean values of fungal load, with 3.79 x 10^5 CFU/g and 2.60 x 10^5 CFU/g, respectively. While the Wednesday Market had the lowest mean value of 1.74 x 10^5 CFU/g.

Table 3: shows that the most prevalent fungal species isolated from the samples was *Aspergillus nidulans*, which appeared in 14 samples with 27.45%, *Rhizopus arrhizus*, which appeared in 13 samples with 25.59%, and fungi that appeared moderately were *Trichophyton interdigitale* and *Aspergillus niger,* which appeared in 8 samples with 15.68% and 6 samples with 11.76%, respectively. *Trichophyton verrucosum* was found in 3.8% of the samples. However, *Aspergillus fumigatus* and *Aspergillus flavus* were found in 3.92% of the samples. Finally, the fungi discovered to be very low were *Aphanoascus fulvescens*, *Aspergillus lentulus,* and *Chaetomium kunze*, which appeared only in one sample, constituted a percentage of occurrence of 1.96%.

Fig. 1. Explains that *Aphanoascus fulvescens, Aspergillus lentulus,* and *Chaetomium kunze* were found to be present only in FUDMA Takeoff Campus Samples. Also, *Aspergillus flavus* was found to be present only in Dutsin-Ma Wednesday Market

Table 1. Cultural and microscopic features of fungal species isolated from powdered groundnut cake

S/N	Cultural Features	Microscopic Features	Identified fungi
1.	Colonies are white to tan with the production of numerous spherical, pseudo parenchymatous, buff to light brown cleistothecia.	Asci are subspherical to ellipsoidal with spores. Ascospores are light brown, yellowish to pale brown in mass, irregularly reticulate.	*Aphanoascus fulvescens*
2.	Colonies are granular, flat, with radial grooves, yellow at first but quickly becoming bright to dark yellow-green.	Conidial heads are typically radiate, biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened. Conidia are globose, pale green and conspicuously echinulate.	*Aspergillus flavus*
3.	Colonies are typically blue-green with a suede-like surface consisting of a dense felt of conidiophores.	Conidial heads are typically columnar and uniseriate. Conidiophore stipes are short, smooth-walled and have conical-shaped terminal vesicles.	*Aspergillus fumigatus*
4.	Colonies are suede-like to floccose, white with interspersed grey-green patches of conidia.	Conidial heads are short, columnar and uniseriate. Conidiophore stipes are smooth-walled, and are constricted at the neck. Vesicles are usually subglobose in shape. Conidia globose to broadly ellipsoidal, smooth to finely roughened.	*Aspergillus Lentulus*
5.	Colonies are plain green in colour with dark red-brown cleistothecia developing within and upon the conidial layer	Conidial heads are short, columnar and biseriate. Conidiophore stipes are short, brownish and smooth-walled. Conidia are globose and rough-walled.	*Aspergillus nidulans*
6.	Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large, biseriate, large globose, dark brown, becoming radiate, biseriate with the phialides borne on metulae.	*Aspergillus niger*
7.	Colonies are moderately fast growing and dark in colour.	Formation of darkly-pigmented, globose, ovoid, barrel to flask-shaped, ostiolate ascocarps beset with dark-coloured terminal hairs. Ascospores are one-celled, darkly-pigmented, smooth-walled, of varying shape, mostly ovoid, ellipsoidal or lemon-shaped.	*Chaetomium kunze*
8.	Colonies are white cottony at first becoming brownish grey to blackish-grey depending on the amount of sporulation.	Sporangiophores are smooth-walled, non-septate, simple or branched, arising from stolons opposite rhizoids in groups of three. Sporangia are globose, with a flattened base, greyish black, powdery in appearance, and many spored. Sporangiospores are angular, subglobose to ellipsoidal, with	*Rhizopus arrhizus*

S/N	Cultural Features	Microscopic Features	Identified fungi
		striations on the surface.	
9.	Colonies are flat, white to cream in colour with a powdery to suede-like surface and yellowish to pinkish brown reverse pigment	Numerous subspherical to pyriform microconidia, spiral hyphae and spherical chlamydospores are present, slender, clavate, smoothwalled, multiseptate macroconidia	*Trichophyton interdigitale*
10.	Colonies are slow growing, small, disk-shaped, white to cream-coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth.	Broad, irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are in chains. The tips of some hyphae are broad and club-shaped,	*Trichophyton verrucosum*

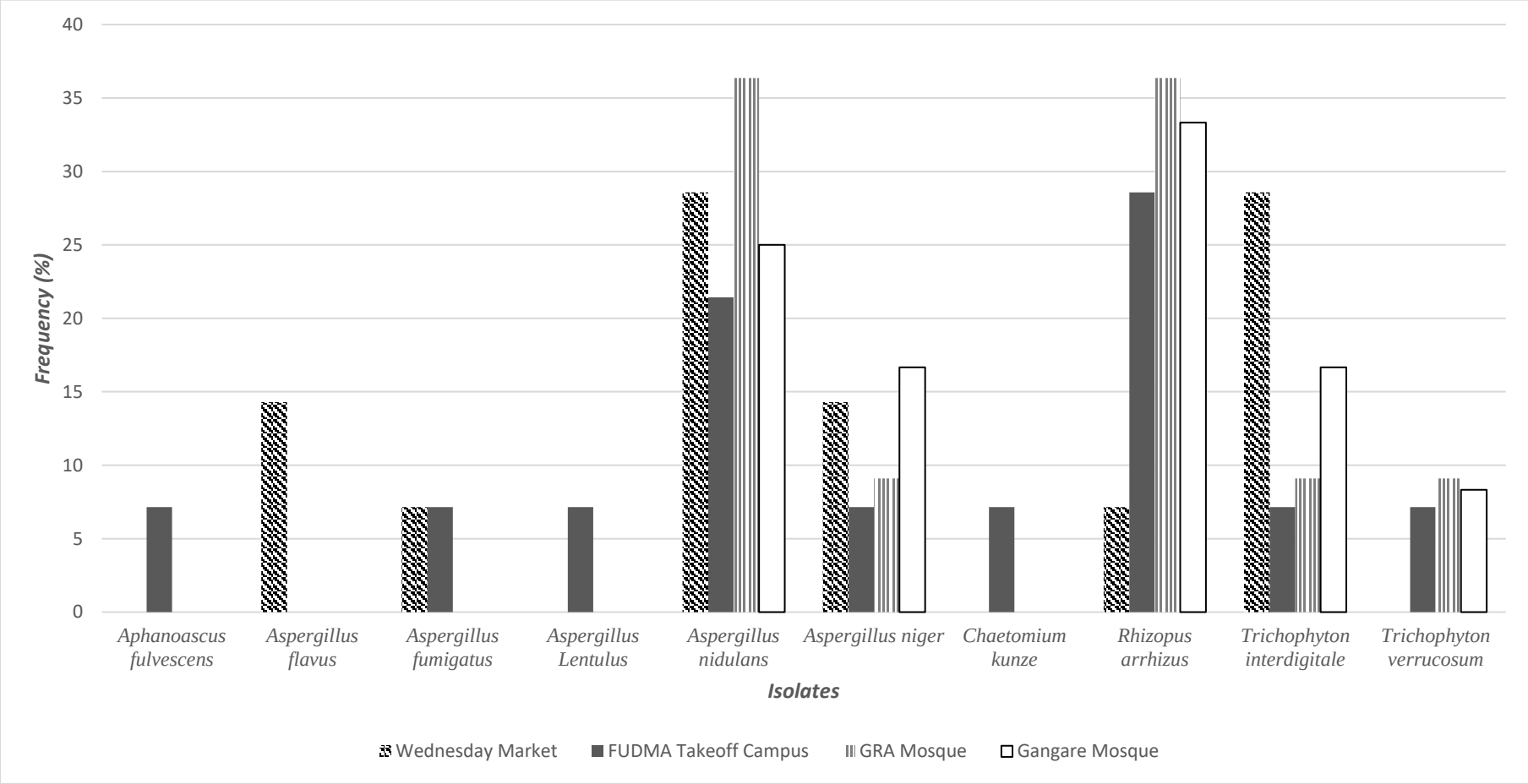

Fig. 1. Prevalence and distribution of fungi isolated from the powdered groundnut cake sample

samples. *Trichophyton verrucosum* was found to be present in nearly all the locations except in Dutsin-Ma Wednesday Market samples. *Aspergillus fumigatus* was found to be present in Dutsin-Ma Wednesday Market and FUDMA Takeoff Campus Samples. *Aspergillus niger* and *Trichophyton interdigitale* were found to be present in all the samples from all locations. Finally, *Aspergillus nidulans* and *Rhizopus arrhizus* were found to be the most prevalent and widely distributed fungi isolated from samples in all locations. This corresponds with the finding of EIT. [14].

4. DISCUSSION

According to the findings of this investigation, groundnut cake samples from GRA Mosque appeared to be more contaminated with harmful fungi than those from FUDMA Takeoff Campus, Gangare Mosque, and Wednesday Market. This is because the greatest count (6.61 x 10^5 cfu/g) was acquired from GRA Mosque, while the lowest count (1.74 x 10^5 cfu/g) was found from Wednesday Market. This is expected because merchants at the Wednesday Market may have more opportunities to be taught on proper hygiene during preparation than vendors at the GRA Mosque. However, there was no significant difference in the fungal load of the various samples studied (analysis of variance= 8.003559, $p<0.05$, F= 2.250131 and df=9).

The population and type of fungi recovered from powdered groundnut cake samples in this study revealed a serious public health risk because some of the species are known toxin producers, while others were saprophytes, causing deterioration of the food material in their attempt to adapt and survive in the microenvironment. The *Aspergillus* and *Rhizopus* species isolated from powdered groundnut cake samples support the findings of Salau et al. [9], who reported comparable fungi as contaminants of groundnut cake, as well as others that were not recovered or could not be identified in this study. According to Jimoh and Kolapo [15]. these fungi are the most common contaminants of groundnut products in storage this aslo agrees with the finding of Harvest Biotechnology [16]. As a result, their presence in this food product could be attributed to the raw groundnut employed in the individual groundnut cake manufacturing as well as postproduction (i.e., exposure of this marketed snack to fungal spores living in the air). Because the fungus count was higher at GRA Mosque than at FUDMA Takeoff Campus, Gangare Mosque, and Wednesday Market locations in Dutsin-Ma, the former may be a small influence compared to the latter (exposure of snacks in markets to airborne fungal spores). The presence of *Aspergillus species* such as *A. flavus, A. fumigatus, A. Lentulus, A. nidulans,* and *A. niger, and Rhizopus* in Powdered Groundnut Cake samples contradicts the findings of many researchers including Akano and Atanda, [17] Jimoh and Kolapo, [15] Makun et al. [17] Salau et al. [9] and further concluded that such isolate Rhizopus has been shown to create the metabolite *rhizonin* [18] and the fungus also make *aflatoxins, ochratoxins, fumonisins, trichothecenes, citrinin,* and *patulin* during metabolism. Following the occurrence of mortality caused by aflatoxin-contaminated foods in Nigeria, Akano and Atanda [17] reported the presence of identical fungi and aflatoxins in groundnut cake from Ibadan, Oyo State, Nigeria. Stored powdered groundnut cake represents a complex ecosystem in which fungus spoilage is determined by a variety of parameters divided into four groups: intrinsic nutritional factors, extrinsic factors, processing factors, and implicit factors (Magan *et al.,* 2004). These factors, alone or in combination, impact the composition of the fungus population, causing variations over the storage period. Oko et al. [19]

The recovery of propagules of fungal species in all powdered groundnut cake samples and the significantly higher incidence of *A. nidulans* species compared to other isolates support previous studies showing that groundnut is highly susceptible to contamination from *Aspergillus species.* Sola et al., Nyirahakizimana et al. [20,21].

Table 2. Mean mold count of powdered groundnut cake samples from four major locations

S/N	Locations	Mean Mould Count (CFU/g)
1.	Wednesday Market	1.74 X 10^5
2.	FUDMA Takeoff Campus	3.79 X 10^5
3.	GRA Mosque	6.61 X 10^5
4.	Gangare Mosque	2.60 X 10^5

Table 3. Percentage of occurrence of fungal species

S/N	Isolates	Frequency	Percentage (%)
1.	*Aphanoascus fulvescens*	1	1.96%
2.	*Aspergillus flavus*	2	3.92%
3.	*Aspergillus fumigatus*	2	3.92%
4.	*Aspergillus lentus*	1	1.96%
5.	*Aspergillus nidulans*	14	27.45%
6.	*Aspergillus niger*	6	11.76%
7.	*Chaetomium kunze*	1	1.96%
8.	*Rhizopus arrhizus*	13	25.49%
9.	*Trichophyton interdigitale*	8	15.68%
10.	*Trichophyton verrucosum*	3	5.88%
	Total	51	100%

5. CONCLUSION

The mycological assessment conducted on powdered groundnut cake from selected areas in Dutsin-Ma, Katsina State, revealed significant insights into fungal contamination and its implications for food safety and public health.

The study identified a diverse range of fungal species present in the sampled powdered groundnut cake, predominantly including species from *Aspergillus, Penicillium, and Fusarium* genera. Quantitative assessments indicated varying contamination levels among different samples, with some exhibiting significantly higher fungal loads compared to others Copetti [22].

Notably, toxigenic fungi, particularly *Aspergillus* species known for their ability to produce aflatoxins, were prevalent in a substantial proportion of the samples.

This finding is of considerable concern due to the potential health risks associated with aflatoxin exposure, including carcinogenic and hepatotoxic effects. As also indicated by Kigigh et al. [23].

Analysis of contributing factors highlighted deficiencies in storage conditions, inadequate processing practices, and suboptimal hygiene standards during production and handling processes. Garnier et al. [24].

Environmental conditions favoring fungal growth, such as high humidity and improper temperature control, were also identified as significant contributors to contamination FAO. [25]

The presence of toxigenic fungi, especially those capable of producing mycotoxins such as aflatoxins, raises serious concerns regarding the safety of powdered groundnut cake sold in these areas Elegbede [26].

6. RECOMMENDATION

1. Implementation of stringent quality control measures throughout the production, processing, and distribution chain is imperative to mitigate fungal contamination.
2. Improved storage facilities, enhanced drying methods, strict adherence to hygiene protocols, and education programs aimed at producers, vendors, and consumers are vital to minimize fungal proliferation and mycotoxin contamination.
3. Compliance with international food safety standards regarding mycotoxin levels in food products is essential to safeguard public health and ensure trade compliance.
4. Future research endeavors should focus on exploring novel technologies for mycotoxin detection, refining production techniques to reduce fungal contamination, and assessing the long-term health impact of consuming contaminated groundnut cake.
5. In conclusion, the findings underscore a critical need for immediate interventions and regulatory measures to address the mycological quality issues identified in powdered groundnut cake sold in Dutsin-Ma, Katsina State. Safeguarding consumer health requires collaborative efforts involving producers, regulatory bodies, and stakeholders across the food supply chain to ensure the production and distribution of safe, high-quality food products free from harmful mycotoxins and fungal contaminants [27-38].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Anova: Two-Factor Without Replication						
SUMMARY	**Count**	**Sum**	**Average**	**Variance**		
Row 1	4	1	0.25	0.25		
Row 2	4	2	0.5	1		
Row 3	4	2	0.5	0.333333		
Row 4	4	1	0.25	0.25		
Row 5	4	14	3.5	0.333333		
Row 6	4	6	1.5	0.333333		
Row 7	4	1	0.25	0.25		
Row 8	4	13	3.25	2.25		
Row 9	4	8	2	2		
Row 10	4	3	0.75	0.25		
Column 1	10	14	1.4	2.488889		
Column 2	10	14	1.4	1.377778		
Column 3	10	11	1.1	2.544444		
Column 4	10	12	1.2	2.177778		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	56.225	9	6.247222	8.003559	1.14E-05	2.250131
Columns	0.675	3	0.225	0.288256	0.833457	2.960351
Error	21.075	27	0.780556			
Total	77.975	39				

Peer-review history:
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