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Survey and Determination of Aflatoxin Levels in Stored Peanut in Sudan

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Abstract

Sixty samples of stored peanut kernels were collected from four different locations in Sudan namely Mayo city, Umbaddah city in Khartoum state, Al-Helalia city, and Al-Managel city in Al-Jazeera state. The kernels were examined for contamination with aflatoxin. All samples were subjected to microbiological analysis by culturing them on suitable growth medium "Sabouraud's Dextrose Agar" and chemical analysis by Thin-Layer Chromatography (TLC) technique. Thirty five samples (58.33%) gave positive readings with TLC technique, and in culture, *Aspergillus flavus* was isolated from twenty six samples (43.33%). The concentration of aflatoxin B_1 in these samples were ranged from low to very high, in range of (17.57-404.00 µg/Kg kernel).

الملخص

أجريت هذه الدراسة بهدف تحديد مدى تلوث الفول السوداني المأخوذ من مناطق عديدة بالسودان بالسم الفطري المعروف بالأفلاتوكسين ب$_1$.
جُمعت ستون عينة من الفول السوداني المخزون من مناطق مختلفة بالسودان شملت ولايتي الخرطوم (مدينة مايو ومدينة أم بدة) وولاية الجزيرة (مدينة الهلالية ومدينة المناقل) بمعدل خمسة عشر عينة من كل مدينة.
كل العينات أختبرت عن طريق التحليل الميكروبي للتعرف على الفطريات المصاحبة وذلك بواسطة الزراعة على وسط أجار السابرود ديكستروز وكذلك عن طريق التحليل الكيميائي بواسطة تقنية التصوير الملون ذات الطبقة الرقيقة لمعرفة ما إذا كانت العينات ملوثة بسم الافلاتوكسين ب$_1$.
إحتوت خمس وثلاثون عينة بنسبة (58.33%) من مجموع عينات الفول السوداني المخزون على سم الافلاتوكسين ب$_1$ عن طريق تقنية التصوير الملون ذات الطبقة الرقيقة. أما في التحليل الميكروبي فقد عُزل فطر (*Aspergillus flavus*) من ست وعشرون عينة بنسبة (43.33%).
تراوح تركيز سم الافلاتوكسين ب$_1$ في العينات المختبرة مابين منخفض، عالي، وعالي جداً في مدى يتراوح ما بين (17.57-404.00 مايكروجرام/كجم) في عينات حبيبات الفول السوداني المخزون، بما يؤكد إرتفاع تلك النسب عن المستوى المسموح وما يمثله من خطورة محتملة للإنسان والحيوان.

Keywords: *Key words*: Aflatoxin, Mycotoxins, Peanuts, *Aspergillus flavus*, TLC.

1. Introduction

Aflatoxin is the name for a group of toxins known as B_1, B_2, G_1, G_2, M_1 and M_2 (carcinogenic compounds) that are produced mainly by two fungi called *Aspergillus flavus* and *Aspergillus parasiticus*. These toxins occur naturally and have been found in a wide range of commodities (including peanuts) used for animal and human consumption. Depending on their levels, toxins can severely affect the liver and induce a human carcinogen, i.e., causes cancer. In many developing countries, aflatoxin is a major health risk to both humans and animals due to the high levels of the contaminated products consumed (Wright *et al*., 2002).

The causative agents grow on food and feed grains at a moisture level of 15% or greater in the presence of warm temperatures (21˚C- 37˚C or 70˚-100˚F). The toxin can be found in a variety of grains but most often occurs in peanut and corn. Contamination can occur while the grain is standing in the field, at and soon after harvesting and during storage before or after the grain is processed into food or feed (Allen, 2003).

A few months after the death of more than 100.000 young Turkeys in poultry farms in England, an apparently new disease that was termed "Turkey X disease" appeared. Speculations made during 1960 regarding the nature of the toxin suggested that it might be of fungal origin. In fact, the toxin-producing fungus was identified as *Aspergillus flavus* in 1961 and the toxin was given the name Aflatoxins by virtue of its origin (*A. flavus* → Afla) (Liu *et al*., 2005).

The chemical structure of aflatoxin is coumarin nucleus linked to a bifuran and either a pentanone, as in AFB_1 and the dihydro derivative AFB_2, or a six – member lactone, as

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in AFG_1 and its corresponding derivative AFG_2 (Sanz *et al*., 1989)

The economic impact of aflatoxins was derived directly from crop, livestock losses, and, , indirectly, from the cost of regulatory programs designed to reduce risks to animal and human health. The Food and Agriculture Organization (FAO) estimates that 25 % of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins. Aflatoxins losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency. Other adverse economic effects of aflatoxins include lower yield for food and fiber crops (Anon, 1989)

In an attempt to harmonize the current tolerances to aflatoxin which exist in different countries, the working group on mycotoxins of the World Health Organization (WHO) and Food Agricultural Organization (FAO) proposed maximum limits of 15µg/Kg for total aflatoxins in raw groundnuts based on a sample size of 20 Kg (Bhat *et al*., 1996).

The potential economic problems associated with a level of 10µg/Kg and the public health implications of a level of 15µg/Kg, as compared to 10µg/Kg for aflatoxins in foods, are two main issues in the setting of maximum levels for aflatoxins in groundnuts intended for further processing. Many countries considered the level of 15µg/Kg to be a reasonable limit that could be achieved by producing countries, thus facilitating international trade, considering that a lower level would constitute a trade barrier due to the finding of the the evaluation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) that this may not offer a significant improvement in public health. However, genotoxic properties of aflatoxin, uncertainties in risk assessment, the As Low As is Reasonable Achievable (ALARA) principle, and inadequate data on the effect of a level of 10µg/Kg on the availability of groundnuts in the world market support the continued consideration of the lower level. A situation can be envisaged from a recent Multi-Centric National Study in India of aflatoxin contamination in maize and groundnut. The study indicated that 21% of the contaminated groundnut samples available in the Indian market were not suitable for human consumption as they contained aflatoxin B_1 above the Indian permissible limit of 30µg/Kg (Bhat *et al*., 1997).

1.1. Objectives

The objectives of this study are to:

1. Isolate the fungi which produce aflatoxin B_1 from stored peanut kernels.
2. Measure the quantity of aflatoxin B_1 in stored peanut kernels.

2. Material and Methods

2.1. Microbiological analysis

Peanut samples were collected from four different locations in Sudan by taking 100 gm from each sample and mixing them to obtain the representative sample. One gram of each sample was tested for fungal isolation by inoculating the Sabouraud dextrose agar plates with the suspension of peanut kernels using dilution method by taking 0.5 ml from each dilution; they were placed into Petri dishes and incubated at 28˚C for 3-5 days (Hayate and Idris, 2000). After the incubation period, the growing fungal cultures were examined microscopically using Lactophenol Cotton Blue (LPCB) stain and classified by reporting the culture characteristics at the face and reverse side of the inoculated Petri dishes (Cheesbrough, 1984).

2.2. Chemical analysis

The chemical analysis techniques were performed according to those described by the Association of Official Analytical Chemist (AOAC, 1999).

2.2.1. Extraction of aflatoxin from peanut kernels

Twenty grams Peanut samples were ground and placed in 250 ml conical flask, 100 ml of methanol (55%), and 40 ml of petroleum ether were added and blended for 2 minutes at a high speed. The mixture was left standing undisturbed in the blender for 30 minutes. Twenty five ml were pipetted from the aqueous methanol phase into 250 ml flask, and 25 ml of chloroform were added, covered with a stopper and shaken for 1 minute. The layers were left to separate and the bottom chloroform layer was drained into 100 ml glass beaker, placed into a water bath to evaporate the solvent. The extract was dissolved in 200µl benzene-acetonitril (98:2) for spotting on TLC plate (AOAC, 1999).

2.2.2. Detection of aflatoxin using Thin-Layer Chromatography(TLC)

20µl from the previously prepared sample was spotted on imaginary line 1cm from the bottom edge of TLC plate and 20µls of the aflatoxin reference standard solution. The plate was placed in a tank containing a mixture of acetone-chloroform (5:95) for 10 minutes at 23-25˚C, removed, allowed to dry at room temperature, and illuminated from above by placing its flat, coated side up, on a long wave ultraviolet lamp Chromato-Vue cabinet. The fluorescent spots were observed and the retention factor (R_f) was calculated as in formula (i), recorded to determine the concentration of aflatoxin B_1 after spraying the plate with 50% sulphuric acid solution. The results were calculated applying formula (ii).

Formula (i)

$$R_f = \frac{\text{distance moved by compound}}{\text{distance moved by solvent}}$$

Formula (ii)

Concentration of aflatoxin B_1 in µ/Kg = (S× Y× V)/ (X× W)

Where:

S ≡ µl aflatoxin B_1 standard equal to unknown.

Y ≡ concentration of aflatoxin B_1standard µg/ml.

V ≡ µl of final dilution of sample extract.

X ≡ µl of sample extract spotted to giving fluorescent intensity equal to S (B_1 standard).

W ≡ weight of sample in gram of original sample contain in final extract.

The R_f of aflatoxin B_1 is ranged between 0.4-0.7(AOAC, 1999).

3. Results

Fifteen stored peanut kernel samples, which were collected from Mayo city, Khartoum state, contained aflatoxin B_1 by using chromatographical technique (Fig.1) and they had a range of aflatoxin B_1 concentration from (17.57 µg/ Kg – 67.33 µg/ Kg Table 1), but not all samples containing the aflatoxin B_1 producing-agents (*Aspergillus flavus*). This fungus was isolated from six samples (40%) only and the others contained *Aspergillus niger*, (Table 2). While eight samples (53.33%) of stored peanut kernels collected from Umbaddah city, Khartoum state, appeared positive with chromatographical technique with toxin concentration ranged from (44.89 µg/ Kg – 404.00 µg/ Kg (Table 3), and nine samples (60%) yielded *Aspergillus flavus*, and the rest samples were contaminated with *Aspergillus niger* (Table 4).

The samples which were collected from Al-Jazeera state, notably from Al-Helalia city, showed positive results to the chemical analysis in seven samples (46.67%) with toxin concentration ranged from (36.70 µg/ Kg – 101.00 µg/ Kg (Table 5). The fungal isolated from these samples showed that only six samples (40%) contained *Aspergillus flavus* and the rest were contaminated with *Aspergillus niger* (Table 6). The last location in Al-Jazeera state is Al-Managel city; from this location five samples (33.33%) showed positive in chemical analysis with toxin concentration, ranging from (25.25 µg/ Kg - 80.80 µg/ Kg (Table 7). These samples were also positive in microbiological analysis and the others contained *Aspergillus niger* (Table 8).

The thirty five samples (58.33%) from all the samples (sixty samples) of stored peanut kernels were contaminated with aflatoxin B_1, and all samples (ten samples) after storage time of twenty four months were positively in the chromatographical technique, but not in microbiological analysis except for one sample. While the 73.33% (11 samples from 18) after twelve months storage time showed positive result for chemical analysis, and 66.67% (10 samples) showed positive to the microbiological analysis. In contrast, 35.71% (10 samples from 28) of six months storage time were positive to the chemical analysis and 39.29% (11 samples) were positive in microbiological analysis. Seventy five percent (3 samples from 4) of two months storage time showed positive in both chemical and microbiological analysis. All samples which contained aflatoxin B_1 were visible after spraying with 50% sulphuric acid as brown spots while the others were detected as yellowish-brown spots.

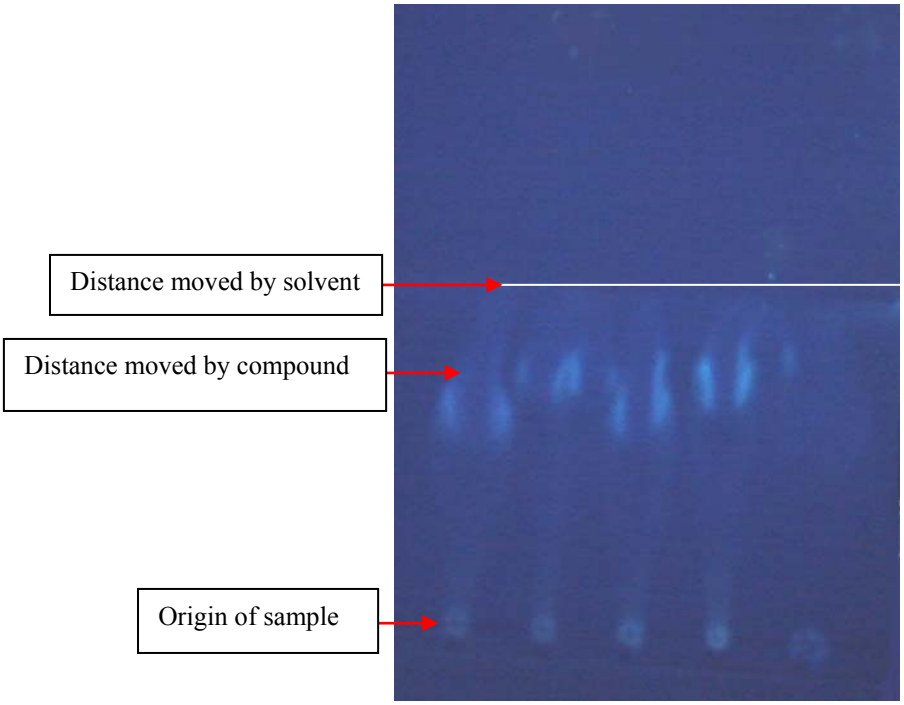

Figure 1. The positive result of aflatoxin B_1 under U.V. light of stored peanut kernels with R_f equal 0.66.

Table **1**. Screening of aflatoxin B_1 (AFB_1) in stored peanut kernels from Mayo city, Khartoum state.

No of sample	Storage time/Months	Weight of extract/g	R_f	Conc. of AFB_1 (µg/Kg)	Appearance under U.V (650 nm wave length)	Visual color
1.	6	0.60	0.61	67.33	Fluorescent	Brown
2.	6	0.80	0.61	50.50	Fluorescent	Brown
3.	6	0.90	o.70	44.89	Fluorescent	Brown
4.	6	0.30	0.67	134.66	Fluorescent	Brown
5.	6	0.60	0.69	67.33	Fluorescent	Brown
6.	24	2.20	o.61	18.36	Fluorescent	Brown
7.	24	1.20	0.66	33.67	Fluorescent	Brown
8.	24	1.90	0.66	21.26	Fluorescent	Brown
9.	24	1.70	0.58	23.76	Fluorescent	Brown
10.	24	1.90	0.45	21.26	Fluorescent	Brown
11.	24	0.80	0.55	50.50	Fluorescent	Brown
12.	24	1.20	0.55	33.67	Fluorescent	Brown
13.	24	1.80	0.55	22.44	Fluorescent	Brown
14.	24	1.90	0.50	21.26	Fluorescent	Brown
15.	24	2.30	0.53	17.57	Fluorescent	Brown

$R_f \equiv$ Retention Fa

Table **2**. Fungi isolated from stored peanut kernels collected from Mayo city, Khartoum state.

No. of sample	Fungi isolated
1.	*Aspergillus flavus*
2.	*Aspergillus flavus*
3.	*Aspergillus flavus*
4.	*Aspergillus flavus*
5.	*Aspergillus flavus*
6.	*Aspergillus niger*
7.	*Aspergillus niger*
8.	*Aspergillus niger*
9.	*Aspergillus niger*
10.	*Aspergillus niger*
11.	*Aspergillus niger*
12.	*Aspergillus niger*
13.	*Aspergillus niger*
14.	*Aspergillus niger*
15.	*Aspergillus flavus*

Table **3**. Screening of aflatoxin B_1 in stored peanut kernels from Umbaddah city, Khartoum state.

No of sample	Storage time/Months	Weight of extract/g	R_f	Conc. of AFB_1 (µg/Kg)	Appearance under U.V (650 nm wave length)	Visual color
1.	12	0.50	0.66	80.80	Fluorescent	Brown
2.	12	0.30	0.70	134.66	Fluorescent	Brown
3.	12	0.40	0.70	101.00	Fluorescent	Brown
4.	12	0.30	0.00	0.00	Fluorescent	Brown
5.	12	0.70	0.66	57.71	Fluorescent	Brown
6.	12	0.60	0.48	67.33	Fluorescent	Brown
7.	12	0.70	0.89	0.00	Fluorescent	Yellow
8.	2	0.90	0.82	0.00	Fluorescent	Yellow
9.	2	0.60	o.78	67.33	Fluorescent	Brown
10.	2	0.90	0.71	44.89	Fluorescent	Brown
11.	2	0.10	0.67	404.00	Fluorescent	Brown
12.	6	1.00	0.00	0.00	Inflorescent	Yellow
13.	6	0.50	0.00	0.00	Inflorescent	Yellow
14.	6	1.00	0.00	0.00	Inflorescent	Yellow
15.	6	0.10	0.00	0.00	Inflorescent	Yellow

$R_f \equiv$ Retention Factor.

Table **4**. Fungi isolated from stored peanut kernels collected from Umbaddah city, Khartoum state.

No. of sample	Fungi isolated
1.	*Aspergillus flavus*
2.	*Aspergillus flavus*
3.	*Aspergillus flavus*
4.	*Aspergillus niger*
5.	*Aspergillus flavus*
6.	*Aspergillus flavus*
7.	*Aspergillus niger*
8.	*Aspergillus niger*
9.	*Aspergillus flavus*
10.	*Aspergillus flavus*
11.	*Aspergillus flavus*
12.	*Aspergillus niger*
13.	*Aspergillus flavus*
14.	*Aspergillus niger*
15.	*Aspergillus niger*

Table **5**. Screening of aflatoxin B_1 in stored peanut kernels from Al-Helalia city, Al-Jazeera state.

No of sample	Storage time/Months	Weight of extract/g	R_f	Conc. of AFB_1 (µg/Kg)	Appearance under U.V (650 nm wave length)	Visual color
1.	6	0.30	0.00	0.00	Inflorescent	Yellow
2.	12	1.00	0.71	40.40	Fluorescent	Brown
3.	12	1.00	o.71	40.40	Fluorescent	Brown
4.	12	1.10	0.71	36.70	Fluorescent	Brown
5.	12	1.10	0.71	36.70	Fluorescent	Brown
6.	12	1.00	0.71	40.40	Fluorescent	Brown
7.	12	0.80	0.66	50.50	Fluorescent	Brown
8.	12	1.00	0.87	0.00	Fluorescent	Yellow
9.	12	1.10	0.87	0.00	Fluorescent	Yellow
10.	12	0.90	0.9	0.00	Fluorescent	Yellow
11.	12	1.30	0.97	0.00	Fluorescent	Yellow
12.	12	1.30	0.97	0.00	Fluorescent	Yellow
13.	6	0.40	0.78	101.00	Fluorescent	Brown
14.	6	0.20	0.00	0.00	Inflorescent	Yellow
15.	6	0.30	0.00	0.00	Inflorescent	Yellow

$R_f \equiv$ Retention Factor.

Table **6.** Fungi isolated from stored peanut kernels collected from Al-Helalia city, Al-Jazeera state.

No. of sample	Fungi isolated
1.	*Aspergillus flavus*
2.	*Aspergillus flavus*
3.	*Aspergillus flavus*
4.	*Aspergillus flavus*
5.	*Aspergillus flavus*
6.	*Aspergillus flavus*
7.	*Aspergillus niger*
8.	*Aspergillus niger*
9.	*Aspergillus niger*
10.	*Aspergillus niger*
11.	*Aspergillus niger*
12.	*Aspergillus niger*
13.	*Aspergillus niger*
14.	*Aspergillus niger*
15.	*Aspergillus niger*

Table **7**. Screening of aflatoxin B_1 in stored peanut kernels from Al-Managel city, Al-Jazeera state.

No of sample	Storage time/Months	Weight of extract/g	R_f	Conc. of AFB_1 (µg/Kg)	Appearance under U.V (650 nm wave length)	Visual color
1.	6	1.00	1.03	0.00	Fluorescent	Yellow
2.	6	1.00	0.71	40.40	Fluorescent	Brown
3.	6	1.60	0.77	25.25	Fluorescent	Brown
4.	6	1.00	0.75	40.40	Fluorescent	Brown
5.	6	1.20	0.81	0.00	Fluorescent	Yellow
6.	6	1.00	0.78	40.40	Fluorescent	Brown
7.	6	0.50	0.69	80.80	Fluorescent	Brown
8.	6	1.30	0.82	0.00	Fluorescent	Yellow
9.	6	1.40	0.88	0.00	Fluorescent	Yellow
10.	6	1.60	0.88	0.00	Fluorescent	Yellow
11.	6	1.70	0.91	0.00	Fluorescent	Yellow
12.	6	0.60	0.88	0.00	Fluorescent	Yellow
13.	6	0.40	0.00	0.00	Inflorescent	Yellow
14.	6	0.20	0.00	0.00	Inflorescent	Yellow
15.	6	0.30	0.00	0.00	Inflorescent	Yellow

$R_f \equiv$ Retention Factor.

Table **8**. Fungi isolated from stored peanut kernels collected from Al-Managel city, Al-Jazeera state.

No. of sample	Fungi isolated
1.	*Aspergillus niger*
2.	*Aspergillus flavus*
3.	*Aspergillus flavus*
4.	*Aspergillus flavus*
5.	*Aspergillus niger*
6.	*Aspergillus flavus*
7.	*Aspergillus flavus*
8.	*Aspergillus niger*
9.	*Aspergillus niger*
10.	*Aspergillus niger*
11.	*Aspergillus niger*
12.	*Aspergillus niger*
13.	*Aspergillus niger*
14.	*Aspergillus niger*
15.	*Aspergillus niger*

4. Discussion

This study demonstrated the wide contamination frequency with aflatoxin in peanut samples from different locations in Sudan. Levels of aflatoxin were high in most locations. These results are similar to those obtained by Lund *et al*. (2000) who reported that the 27 samples (23.5%) of peanut and peanut products of one hundred and fifteen (27/115) showed positive to aflatoxin B_1 with a range of (1.6 – 26.0 µg/ Kg). This range is similar to the twenty two samples (18.33%) of the one hundred and twenty samples of peanut and peanut butter studied in this study. Also Suliman *et al*. (2007) reported that the 73/145 (50%) stored peanut kernels showed positive to aflatoxin B_1 with ranges of (0.8 – 547.5 µg/ Kg); this resembles the range shown in our study (13.47 – 404.00 µg/ Kg).

A survey done in Philippines on peanut-based products revealed that 60% of the samples were positive for aflatoxin B_1 in range of 1.00 - 244 µg/ Kg (Ali *et al*., 1999). In addition, there are several surveys that show a relatively lower level of contamination of aflatoxin B_1 in peanuts and their products. Siame *et al*., (1998) who did a study in Botswana, Africa, reported that the levels of aflatoxin B_1 in a range of (0.8 – 16.00 µg/ Kg) for the raw shelled peanut samples and for peanut butter were (3.2 – 16.00 µg/ Kg). In Tokyo, Japan, Tabata *et al*., (1993) found that several peanut products were contaminated by aflatoxin B_1 in a range of (0.4 – 21.7 µg/ Kg).

According to Ali *et al*. (1999), when the initial content of aflatoxin was high in the raw shelled peanut, a high level of aflatoxin contamination can be expected in its final products such as peanut candy and peanut butter. On the other hand, the low level of aflatoxin contamination in the peanut products has always been associated with the use of high quality raw materials (raw shelled peanut) that contain an acceptable low initial level of aflatoxin. Besides, various peanut processing techniques, such as shelling, drying under sunlight, boiling with salty water, and roasting, were also found to be useful in reducing the aflatoxin content in the products (Yazdanpanah *et al*., 2005).

The percentage of samples (53.33%) for the occurrence of aflatoxin B_1 in this survey was almost similarr to that in several previous studies such as Ali, (2000) who reported the contamination of aflatoxin B_1 in 56% of raw peanut samples, 50% of peanut butter samples, and 50% of other peanut products samples.

5. Conclusion

The levels of aflatoxin B_1 contamination in stored peanut kernel samples, collected from Mayo city, Umbaddah city, Al-Helalia city, and Al-Managel city in Sudan were very high and exceeded the maximum permitted levels according to the WHO/FAO food regulations of 1996, but not all the samples that showed positive results to chemical analysis contained *Aspergillus flavus*.

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