Global systematic review and meta-analysis on prevalence and concentration of aflatoxins in peanuts oil and probabilistic risk assessment

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Submission date: 09-Mar-2023 10:12AM (UTC+0800)

Submission ID: 2032598321 **File name:** 17.pdf (4.37M)

Word count: 6272

Character count: 33148

Review

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Global systematic review and meta-analysis on prevalence and concentration of aflatoxins in peanuts oil and probabilistic risk assessment

34 https://doi.org/10.1515/reveh-2022-0075 Received May 7, 2022; accepted August 9, 2022; published online

Abstract: Exposure to mycotoxins in food is largely unavoidable, and concerns about their health effects are growing. Consumption of vegetable oils such as peanuts oil has increased, hence several studies have been conducted on concentration of aflatoxins (AFs) in peanuts oil. Search was performed in Scopus and PubMed databases on prevalence and concentration of AFs in peanuts oil from 1 January 2005 to 15 April 29, 2022. Prevalence and concentration of AFs in peanuts oil was meta-analyzed based on 7 untry and type of AFs subgroups. In addition, health risk was calculated using monte carlo simulation method. Pooled prevalence of AFB1 in peanuts oil was 47.9%; AFB2, 46.45%; AFG1, 46.92% and AFG2, 54.01%. The Overall prevalence of AFTs was 49.30%, 95%CI (35.80-6231%). Pooled concentration of AFB1 in peanuts oil was 2.30 µg/g; AFB2, 0.77 μg/kg; AFG1, 0.07 μg/kg; AFG1, 0.28 μg/kg. The sort of country based on mean of MOEs in the adults consumers was Japan (47,059) > China (17,670) > Ethiopia (7,398) > Sudan (6,974) > USA (1,012) and sort of country based on mean of MOEs in the children was Japan (120,994) > China (46,991) > Ethiopia (19,251) > Sudan (18,200) > USA (2,620). Therefore, adults consumers were in considerable health risk in Ethiopia, Sudan and USA and for children in USA (MOE < 10,000).

Keywords: aflatoxins; meta-analysis; mycotoxins; peanuts oil; probabilistic risk assessment; vegetable oil.

Introduction

Environmental contamination [1–7] and following food contamination and food sequentially is a severe global health problem [5, 8–15]. Among mycotoxins, aflatoxins (AFs) are one of the secondary and highly toxic metabolites produced by Aspergillus flavus and Aspergillus parasiticus through a polyketide mechanism [16–19]. The four naturally occurring aflatoxins are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), all of which contain the difuran ring and coumarin in their original structure [20–22].

International Agency for Research on Cancer (IARC) classifies AFB1 as one of the most abundant AFs and group I carcinogens [23]and also European Commission in 2002 set AFB1 levels below 20–50 µ₄₀ g in natural feed [24]. These toxins are known to be carcinogenic, teratogenic, mutagenic and immunosuppressive and pose a threat to animal and human health [25–30].

rine growth of aflatoxin-producing fungi and level production of toxins in food is related to fungal species, composition of the food, the percentage of active water, temperature and relative humidity [31].

The lethal dose of aflatoxin (LD_{50}) is 1–50 mg/kg for most 17 imal species and highly toxic effects (LD_{50}) less than 1 mg/kg) for other species such as cats, pigs, and dogs [32].

The exposure to AFs, especially aflatoxin B1, can lead to aflatoxicosis. While constant exposure to AFs can lead to further suppression of cancers, immune responses, and other health effects. The lipophilic structure of AFs facilitates their entry into the bloodstream through the gastrointestinal tract and respiratory tract [27, 33]. About 70 to 90

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percent of liver cancers and also third leading cause of death is related to AFs [27]. It is attributed to liver carcinoma (HCC) with cancer [34]. In this regard, the incidence of liver cancer; is higher in developing countries [27].

Peanut oil is the fifth sost widely used oil in confectionery, candy and pastry. Peanut seeds have high nutritional and commercial value of their protein, fatty acids, carbohydrates and fiber, in addition to vitamins, calcium and phosphorus [35]. Over the past 20 years, the demand for 13 vegetable oils has increased from 72 million tone in 1995 to 17.5 million in 2015 [36]. Global production of peanut oil was 6.48 million tons in 2021 [37]. China and India are the world's largest markets for peanuts and their derivatives (especially peanuts oil) in terms of production and consumption [38].

Experimental studies have shown that aflatoxins present in oily substances can be transferred to the final oil product [39]. The European Commission recommended a maximum level of AFB1 and total against of 2 µg/kg and 4 µg/kg, respectively [29, 40]. The Food and Drug Administration (FDA) has recommended a maximum of 20 µg/kg total AFs in peanuts. U.S. regulations also set a maximum acceptable limit of total contamination of AFs in peanuts of 20 µg/kg.

Crude grains that are used for edible oil are often stored in ina 47 opriate conditions for a long time, thus providing the basis for the growth of fungi and then production mycotoxins and following mycotoxins is eventually transferred to the extracted oil [41]. Therefore, the possible presence of AFs in oilseeds is inevitable, and AFs contamination remains an acute problem, especially in areas with high humidity and rainfall levels [27, 29, 42]. It is difficult to prevent or control peanut contamination by poisonous fungi because the danger of fungi occurs naturally [27, 29, 42].

Many investigation have been conducted on prevalence and concentration of AFs in peanut oil [20, 43–47] but not conducted meta-analysis and pabilistic health risk assessment study. Therefore, the current study was performed with the aim of meta-analysis of the prevalence and concentration of AFs in peanut oils and also probabilistic health risk assessment in consumers of peanut oil content AFs.

Material and method

Search strategy

The retrieve of articles was based on Preferred Reporting Items for Systematic Reviews (Figure 1) [48, 49]. The search was conducted in Scopus and PubMed on Prevalence and concentration of AFs in peanuts oil from 1 January 2005 to 15 April 29, 2022. Keywords were consisting of "mycotoxin" OR "Aflatoxins" OR "Ochratoxin A" OR "patulin" OR "fumonisins" OR "zearalenone" OR "nivalenol/deoxynivalenol" AND "Cereal" OR "peanuts oil" OR "goober-pea" OR "Arachis hypogaea" OR "puny" OR "seed". Disagreement between the authors in selecting an paper was resolved by the corresponding author.

Inclusion, exclusion criteria and data extraction

Our criteria for inclusion paper were present positive sample size or mean with range concentration of AFs; measured of concentration of AFs in peanuts oil and descriptive study. In addition, letter to editors; review articles, chapter; books, conferences, and studies measured concentration of AFs in other vegetables oil were excluded. Country, type of vegetable oil, total sample size, positive sample, type of aflatoxins, mean, SD, Range concentration of AFs, LOD and Method of analysis were extracted (Table 1).

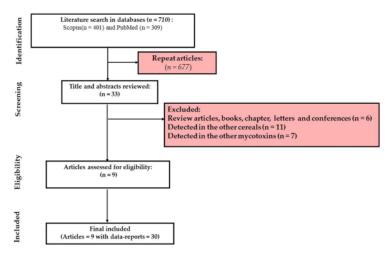


Figure 1: Process selection of study based on PRISMA.

Table 1: Main characteristic included in our study.

37								
Country	Total	Positive	Type of	Mean,	Range,	LOD,	Method	References
	sample size	sample	mycotoxin	$\mu g/kg \pm SD$	μg/kg	µg/kg		
China	8	5	B1	0.50 ± 0.20	0.03-2.23	0.05	55 HPLC-MS/MS	[43]
52 na	8	3	B2	0.50 ± 0.20	0.03-2.23	0.02	HPLCMS/MS	[43]
Ethiopia	5	5	B1	27.28 ± 10.30	0.07-145.59	0.05	HPLC-MS/MS	[43]
Ethiopia	5	5	B2	27.28 ± 10.30	0.07-145.59	0.02	HPLC-MS/MS	[43]
Ethiopia	5	5	G1	27.28 ± 10.40	0.07-145.59	0.09	HPLC-MS/MS	[43]
Ethiopia	5	5	G2	27.28 ± 11.00	0.07-145.59	0.04	HPLC-MS/MS	[43]
Sudan	21	11	B1	16.30 ± 12.00	2.9-36.1	1.5	HPLC-FLD	[20]
Sudan	21	14	B2	1.00 ± 0.50	0.02-3.6	0.09	HPLC-FLD	[20]
Sudan	21	20	G1	12.90 ± 0.50	3.1-64.1	0.8	HPLC-FLD	[20]
20 an	21	19	G2	11.60 ± 0.50	0.5-60.7	0.4	HPLC-FLD	[20]
China	52	43	B1	0.93 ± 0.50	0.5-69.4		UPLC-MS/MS	[44]
Japan	8	5	B1	0.62 ± 0.50	0.52-0.72			[50]
Japan	8	5	B2	0.16 ± 0.50	0.09-0.22			[50]
Japan	8	5	G1	0.08 ± 0.50	0.07-0.08			[50]
Japan	8	5	G2					[50]
China	427	47	B1	29.40 ± 44.40	15.4-49.9	1.08	ELISA	[45]
China	427	33	B1	17.10 ± 26.00	8.8-22.2	1.08	ELISA	[45]
China	31	15	B1	1.29 ± 0.32	0.15-2.72	0.05	LC-MS/MS	[46]
China	31	6	B2	0.11 ± 0.03	0.15-0.36	0.02	LC-MS/MS	[46]
China	31	3	G1	0.02 ± 0.00	0.01-0.02	0.09	LC-MS/MS	[46]
USA	3	1	B1	17.00 ± 8.30		0.03	LC-MS/MS	[47]
USA	3	1	B2	3.00 ± 0.75		0.03	LC-MS/MS	[47]
USA	3	0	G1	0.02 ± 0.01		0.03	LC-MS/MS	[47]
8 A	3	0	G2	$\textbf{0.04} \pm \textbf{0.01}$		0.06	LC-MS/MS	[47]
China	96	22	B1	38.74 ± 47.75	0.26-283.0	0.1	HPLC-FLD	[51]
China	15		B1	0.70 ± 0.40		0.02	HPLC-FLD	[52]
China	120	84	B1	10.94 ± 5.46	0.02-21.85			[52]
China	120	48	B2	1.75 ± 0.87	0.01-3.49		UHPLC-TMS	[52]
China	120	17	G1	0.17 ± 0.08	0.01-0.32		UHPLC-TMS	[52]
China	120	13	G2	0.26 ± 0.12	0.01-0.5		UHPLC-TMS	[52]

Meta-analysis of prevalence and concentration

Meta analysis of prevalence was conducted using "metaprop" command with weighing model of Dersimonian–Laired [53] and also concentration of AFs in peanuts oil was meta-analyzed using average and standard error (SE) [54]. The I-square test used to heterogeneity analysis [55]. Stata 14 (Stata Corporation, College Station, USA) was used to meta-analysis of prevalence and concentration of AFs in peanuts oil.

Probabilistic risk assessment

The health risk in consumers due to the consumption of peanuts oil context of AFs was estimated.

Estimated daily intake was calculated using below equation:

$$EDI = \frac{C \times IR \times \times ED}{BW \times AT}$$
 (1)

where, EDI is estimated daily intake (μg/kg-d), *C* is cor 29 tration of aflatoxins; IR, ingestion rate of peanuts oil (Table 2) [56]; ED, exposure duration (30 years); EF, exposure frequency (350 days/y), BW, body

weight (children: 15 kg and adults: 70 kg) [57] and AT, average life time (for the both adults and children: 25,550 days).

The margin of exposures in consumers due to AFs was calculated by Equation (2) [60]:

$$MOEs = \frac{BMDL10}{EDI}$$
 (2)

In this equation, MOEs is margin of exposure; $BMDL_{10},$ value benchmark dose limit (µg/kg-d). The $BMDL_{10}$ for AFs is equal to

Table 2: Consumption rate of vegetable oil and peanuts oil (kg/n-d).

Country	Vegetable oil [58]	Peanuts oil ^a	
	Mean	Mean	SD
USA	30.600	0.612	0.077
Japan	15.600	0.312	0.039
Ethiopia	3.100	0.062	0.008
Sudan	5.500	0.110	0.014
China	8.100	0.162	0.020

^aConsumption rate of Peanuts oil was almost 2% of vegetable oil [59].

 $0.4 \mu g/kg$ -bw [61]. If, MOEs < 10,000, health risk is consice 28 ble [60–62]. In order to decreased uncertainties in risk assessment, monte carlo simulation (MCS) method was used in Oracle Crystal Ball software (ver. 11.1.2.4). Mean of MOE was considered as benchmark of risk with 50,000 reputations. Lognormal distribution for both concentration and ingestion rate and normal distribution for body weight were considered in MCS method [63, 64].

Results and discussion

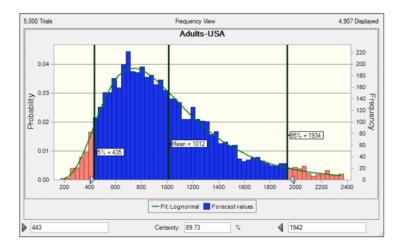
Results of meta-analysis

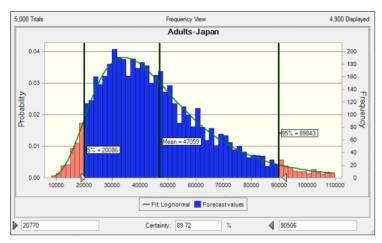
The meta-analysis of aflatoxins in peanuts oil (9 articles with 30 data-reports) (Figure 1) revealed pooled prevalence of AFB1 in peanuts oil was 47.9%, 95%CI (26.96-69.19%); AFB2, 46.45%, 95%CI (30.06 68.91%); AFG1, 46.92%, 95% CI (10.08-85.52%); and AFG2, 54.01%, 95%CI (5.38-98.38%) (Appendix 1). Overall prevalence of total AF was (35.80–62.84%) (Appendix 1). Pooled concentration of AFB1 in proposed nuts oil was 2.30 μg/kg, 95%CI (1.94–2.65 μg/g); AFB2, 0.77 μg/kg, 95%CI (0.58–0.96 μg/ kg); AFG1, 0.07 μg/kg, 95%CI (0.03-0.11 μg/kg); AFG1, 0.28 μg/kg, 95%CI (0.03–0.52 μg/kg). As can be seen from the results, the prevalence and concentration of aflatoxins in peanuts was different though not significant. In the current study, the pooled recentration of AFB1 in peanuts oil was greatly lower than maximum levels (MLs) of 2–8 µg/kg for AFB1 and 15 µg/kg for total aflatoxins that was established by the European Commission [65]. As mentioned from studies, the differences observed in AFs concentration in peanuts oil can be correlated with several factors such as oilseeds species, weather situations, management of seeds and chemical properties of each of produces (moisture, pH, temperature). Also, the AFs can be entered into oil crops during the managing condition post- and preharvest of the product, processing conditions and storage [66, 67]. Various studies have surveyed the concentration of aflatoxins in peanut oil. For ample, Fang et al. (2022) reported from 120 peanut oil sample, the prevalence of AFB1, AFB2, AFG1, and AFG2 was 70.0, 40.0, 14.2 and 10.8%, respectively [65]. Among different edibles oil studied by these authors, peanut oil had the most contamination. They indicated type of packaging and also sampling strategies were effectively affecting the 128 ncentration of AFs [65]. In another study, Chen et al. reported that the average AFB1 and AFs contamination in peanut oil of Ethiopian was 76.98 and 27.28 μg/kg, respectively [68]. Deng et al. showed frogramong 52 peanut oil samples, contamination of AFB1 were detected in 43 samples with the content in the ranges of 0.5–69.4 µg/kg [44]. Furthermore, in similar study Qi et al. found that the average and

range of AFB1 in peanut oil was 29.4 μg/kg [69]. Moreover, Elzupire et al. indicated the mean level of AFT in peanut oil samples was 32.0 μg/kg [20]. In conducted by Yang et al. the levels of AFB1, AFB2 and AFG1 in peanut oils ranged 0.15-2.72, 0.15-0.36 and 0.01-0.02 µg/kg, respectively [46]. According to these investigations, excessive discrepancy between AFT level in peanut oils in our study and those of previous studies may be associated to the species and quality of seeds and also time harvest of crops. The grain quality can be related with different factors such as physical-chemical properties (oxygen, moisture and temperature), the quantity of fungal contamination and duration of storage [70]. Elzupir et al. indicated the main source of AFs contamination in peaget oils is often utilize the oil seeds with low quality that stored over prolonged periods at high temperature and humidity conditions [20]. Contamination of Peanut with AFT at the pre-harvest during may happen because of the collision of the crops with the contaminated soil with fungal. Even the harvest season of the crops can effect on concentration of peanuts to AFs contamination [71, 72]. Consistent with these studies, Qi et al. showed during the harvest stage of peanut seeds, they may they may be stored by rural households and oil processors in an open pile and/or in nylon bags, can easy result to fungal infection [73]. Storage conditions of peanuts are another important factor effective in the observed changes on AFs contamination in peanut oils. According to the investigation conducted by Idris et al. AFs contamination in the peanut oils can be as a result of the unprincipled storage of the seeds in suitable conditions for fungal production and growth [74]. Among other significant issues, several process techniques have key influence on the AFs concentration in oilseed. According to a results obtained by Bordin et al. processes methods such as scaling, heat treatment, wet and dry milling, neutralization, bleaching and deodorization have a many effect on the content of AFs in the peanut oils [70].

Prevalence and concentration of aflatoxins in peanut oil

Based on the obtained data, the sort of country based on prevalence of AFB1 in peanuts oil was Ethiopia (100%) > Japan (62.5%) > Sudan (52.38%) > China (40.48%) > USA (33.33%) (Appendix 2), for AFB2 was Ethiopia (100%) > Sudan (66.67%) > Japan (62.5%) > USA (33.33%) > China (31.76%) > (Appendix 3), in regarding AFG1 was Ethiopia (100%) > Sudan (95.24%) > Japan (62.5%) > China (12.96%) > USA (0.00%) (Appendix 4), and also AFG2 was Ethiopia (100%) > Sudan (90.48%) > Japan





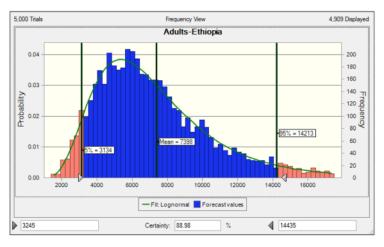
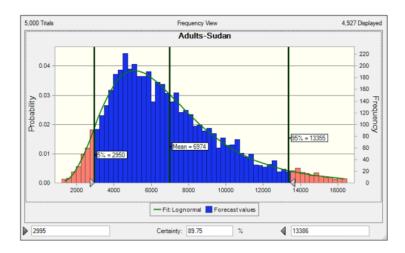


Figure 2: MOE in adults due to consumption peanuts oil content of aflatoxins.



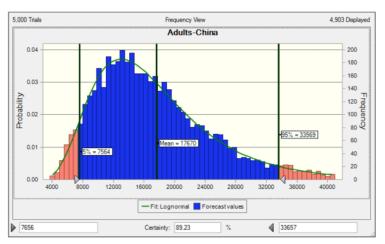
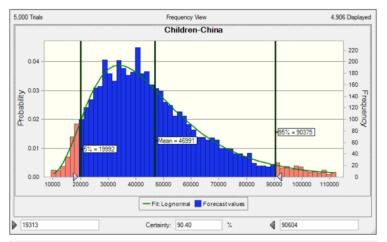


Figure 2: Continued.

(62.5%) > China (10.83%) > USA (0.00%) (Appendix 5). The sort of country based on concentration of AFB1 in peanuts oil was Ethiopia (27.28 μg/kg) > USA (17.00 μg/kg) > Sudan (16.30 μg/kg) > China (4.29 μg/kg) (Appendix 6). The sort of country based on concentration of AFB1 in peanuts oil was Ethiopia (27.28 μg/kg) > USA (3.00 μg/kg) > Sudan (1.00 μg/kg) > China (0.78 μg/kg) > Japan (0.16 μg/kg) (Appendix 7). The sort of country based on concentration of AFG1 in peanuts oil was thiopia (27.28 μg/kg) > Sudan (12.90 μg/kg) > China (0.09 μg/kg) > Japan (0.08 μg/kg) > USA (0.02 μg/kg) (Appendix 8). The sort of country based on concentration of AFG2 in peanuts oil was Ethiopia (27.28 μg/kg) > Sudan (11.60 μg/kg) > China (0.25 μg/kg) > USA (0.04 μg/kg) (Appendix 9). Based on the results, there is a significant difference between various countries

in terms of contamination to aflatoxins. Comparisons between several countries showed that the level of AFs contamination varied in different countries and regions. Oyedele et al. and Kabak reported, in Europe (26.7%) of samples were contamination with AFs in range from 0.6 to $\frac{26}{100}$ 6 µg/kg. In Canada, only 8.3% of samples had AFs ranging from 0.1 to 28 µg/kg. On the contrary, it was seen that the high AFs level in samples were related to other Asiatic countries [75, 76]. According to these studies, AFs content is significantly dependent on geographical position of peanut producers. As mentioned in previous studies in the poorest regions and developing countries in the world with humid and warm weather conditions usually AFs concentration increase because of post-harvest fungal growth [77]. Similarly, Kumar et al. showed in warm and



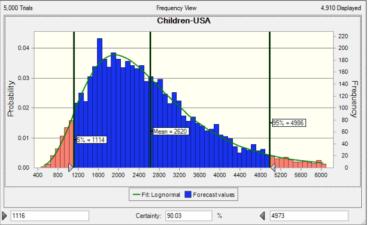


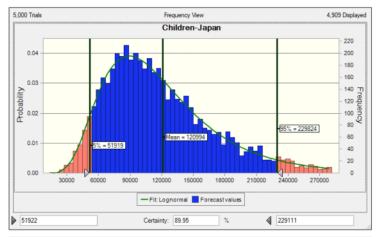
Figure 3: MOE in children due to consumption peanuts oil content of aflatoxins.

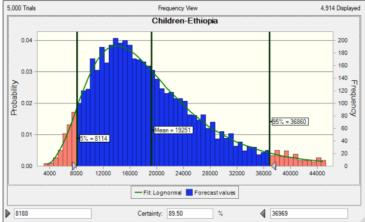
wet climate condition, was more favorable growth of the fungal. In this status to decrease the AFB1 content, recommended for farmers to harvest the raw peanut in good weather condition such as (i.e. sunshine, less rain and fog) and crops should process and story under appropriate temperature and humidity [78]. Besides, hot temperatures and high humidity are the main reasons of fungal growth in subtropical and tropical areas, [71]. Other studies have shown that the species of fungus that infects peanuts and as well as agricultural methods has an important role in the variable content of aflatoxins in peanut oil. Ismail et al. indicated aflatoxigenic fungi, particularly A. Parasiticus and A. flavus have strong affinity for contaminate peanut [79]. Nabizadeh et al. stated that the occurrence of AFs in edible oils in Iran country may be related to different agricultural methods and techniques and also environmental conditions [80]. Similar to these findings, in studies conducted by Chen et al. on samples of peanut oils from

chiopia and China, they reported which oils samples from Ethiopia were more highly contaminated than the Chinese samples possibly due to traditional agricultural, lower awareness among farmers, climatic conditions, late harvesting, and inadequate drying of oil seeds and peanuts. Moreover, authors stated low level of contamination in peanut oils from China can be related to modern technologies, strong agricultural policies and chemical analyses [43]. In line to these studies, Qiu and Fu (2012) reported all positive samples of peanut oil were lower than the regulatory limit, which was attributed to technologies used by alkali-refining processing for AFB1 removal [81].

Risk assessment

The sort of country based on mean of MOEs in the adults consumers was Japan (47,059) > China (17,670) > Ethiopia





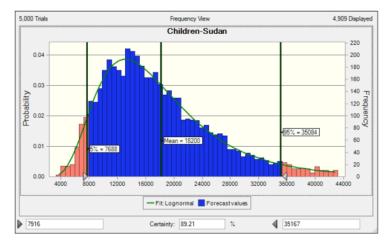


Figure 3: Continued.

(7,398) > Sudan (6,974) > USA (1,012) and in children Japan (120,994) > China (46,991) > Ethiopia (19,251) > Sudan (18,200) > USA (2,620) (Figures 2 and 3). When MOEs value less than 10,000, health risk is considerable [60-62], hence MOE was less than 10,000 for adults in Ethiopia, Sudan and USA and for children in USA.

Conclusions

This study was designed with the aim of meta-analysical prevalence and concentration of AFs in peanut oil and also the health risk in the adults and children consumers were estimated using MCS method. The sort of AFs in peanuts oil based on pooled concentration was AFB1 > AFB2 > AFG2 > AFG1. AFs in approximately 50% of the peanut oil

samples was observed, hence control plans are recommended to reduce the concentration of AFs in peanut oil. The probabilistic health risk assessment revealed that adults consumers in Ethiopia, Sudan and USA and also children in USA were in considerable health risk.

Research funding: This work is supported by the prmozgan University of Medical Science.

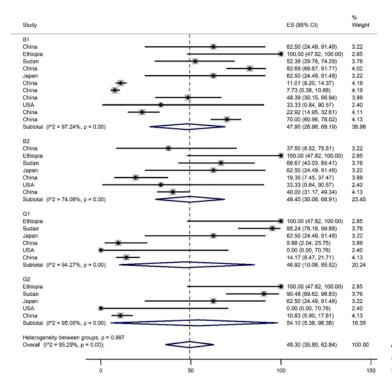
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

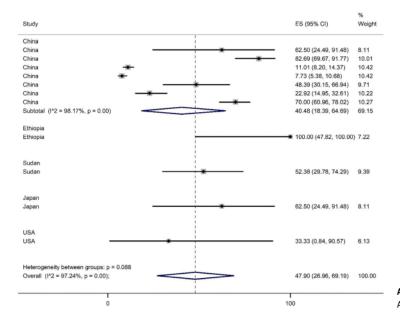
Informed consent 23 ot applicable.

Ethical approval: The conducted research is not related to either human or animal use.

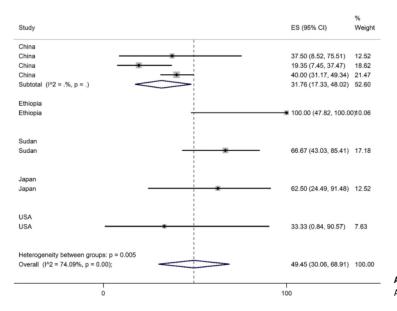
Appendix figures



Appendix 1: Meta-analysis of prevalence of aflatoxins in peanuts oil based on county subgroups.



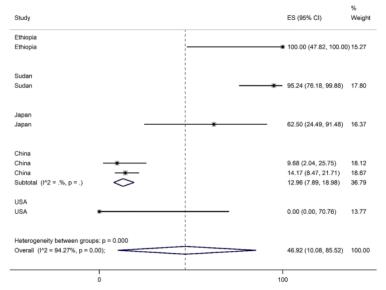
Appendix 2: Meta-analysis of prevalence of AFB1 in peanuts oil.



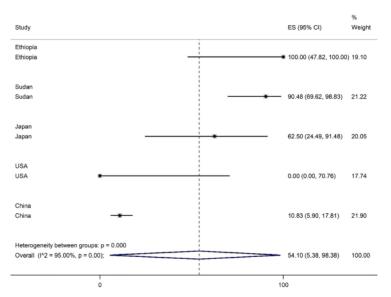
Appendix 3: Meta-analysis of prevalence of AFB2 in peanuts oil.

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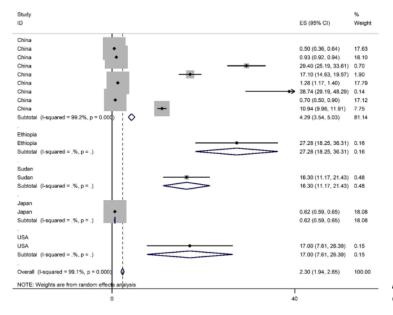
Fakhri et al.: Prevalence and concentration AFs in peanuts oil - 11



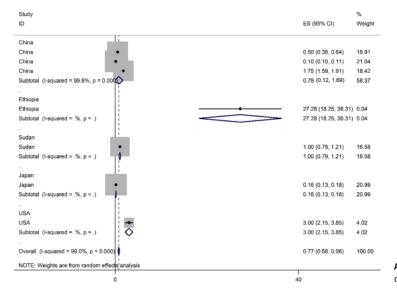
Appendix 4: Meta-analysis of prevalence of AFG1 in peanuts oil.



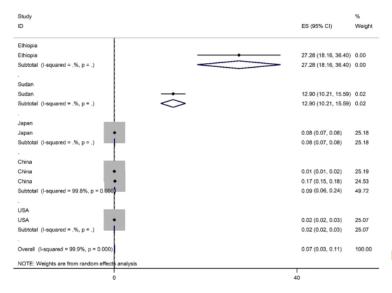
Appendix 5: Meta-analysis of prevalence of AFG2 in peanuts oil.



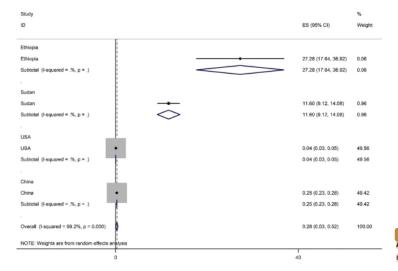
Appendix 6: Meta-analysis of concentration of AFB1 in peanuts oil ($\mu g/kg$).



Appendix 7: Meta-analysis of concentration of AFB2 in peanuts oil ($\mu g/kg$).



Appendix 8: Meta-analysis of concentration of AFG1 in peanuts oil (μg/kg).



Appendix 9: Meta-analysis of concentration of AFG2 in peanuts oil (μ g/kg).

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