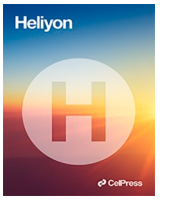
Heliyon 10 (2024) e35023

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Factors influencing aflatoxin B1 levels in the groundnut (*Arachis hypogaea* L.) germplasm of Ethiopia

Yonas Syraji [a], Jeyaramraja PR [b,*], Teklu Wegayehu [a]

[a] Department of Biology, College of Natural and Computational Sciences, Arba Minch University, Arba Minch, Ethiopia
[b] PG and Research Department of Botany, PSG College of Arts & Science, Coimbatore, India

ARTICLE INFO

Keywords:
Aflatoxin B1
Arachis hypogaea
Aspergillus flavus
Groundnut
Kernel colonization

ABSTRACT

As there was no maximum permissible limit prescription for aflatoxin B1 (AFB1) in Ethiopia, this study has been conducted to generate data on AFB1 levels in Ethiopian groundnut accessions/landraces. Besides, an attempt was made to find out if there is any relationship between AFB1 and other parameters such as altitude of cultivation, individual seed weight, kernel colonization by *Aspergillus flavus*, total carbohydrates, protein and total free amino acids. Out of the 28 accessions studied, merely six accessions registered ≤2 ppb AFB1 and thus, they comply with maximum permissible limit set by European Union. Altitude of cultivation had no relationship with AFB1 levels. Interestingly, total carbohydrates in the seeds as well as kernel colonization by *A. flavus* showed statistically significant ($p < 0.01$) positive relationships with AFB1 levels. It is suggested to use kernel colonization measurement as an alternative to the expensive ELISA based AFB1 measurement. Besides, suitable pre- and post-harvest aflatoxin management strategies should be developed to alleviate the AFB1 levels in Ethiopian groundnut.

1. Introduction

Groundnut, a vital oilseed crop in Ethiopia, is cultivated there under rainfed conditions. It is mainly used to obtain cooking oil and to manufacture confectionaries in Ethiopia [1]. This crop is environment friendly as it is a leguminous crop that performs biological nitrogen fixation with the help of *Rhizobia* bacteria in its root nodules. Oromia region leads in groundnut production in Ethiopia, followed by Benshangul-Gumuz and Amhara regional states. Between 2005 and 2014, groundnut production in Ethiopia surged by 72 %, primarily attributed to a 58 % rise in land allocation for cultivation [2]. This crop is prone to infection by *Aspergillus flavus* and *A. parasiticus* which produce a fungal toxin, named aflatoxin which is regarded as a "hidden poison" by Ref. [3] because it disrupts important biological processes in humans, leading to gradual but harmful consequences. There are various types of aflatoxins such as aflatoxin B1 (AFB1), aflatoxin B2, aflatoxin M1 aflatoxin M2, aflatoxin G1, aflatoxin G2, aflatoxicol and aflatoxin Q1 [4].

AFB1, an economically important mycotoxin, is a hepatacarcinogen [5]. Besides being hepatotoxic, AFB1 is also known for its genotoxicity, carcinogenicity, immuno-toxicity and teratogenicity [6]. AFB1 is usually present in groundnut grown under rainfed conditions. The correlation between drought conditions and preharvest aflatoxin contamination in groundnut has been extensively reviewed earlier [7]. Prolonged intake of even small quantities of this mycotoxin can result in the development of liver cancer. When AFB1 contaminated feed is ingested by mammals, AFB1 gets transformed into aflatoxin M1 and M2 which then are excreted in the milk; thus, even newborns can get affected [8]. Continued exposure to aflatoxins has been linked to diminished immune function,

* Corresponding author.
E-mail address: jeyaramrajapr@psgcas.ac.in (J. PR).

https://doi.org/10.1016/j.heliyon.2024.e35023
Received 15 February 2024; Received in revised form 16 June 2024; Accepted 22 July 2024
Available online 22 July 2024

hepatitis B and C infection, undernourishment, growth impairment in children, disabilities, and mortality [9–11]. In low-income countries, there is a problem in deciding what should be given priority, food security or food safety [12].

The market for groundnut in Ethiopia was reported to be on the decline, and the export of the crop was reduced due to the difficulty in meeting the limits of aflatoxin levels set by the importing countries [13]. Several studies were conducted to learn the aflatoxin levels in Ethiopian groundnut [14–17]. In all these studies, the relationship of AFB1 level with other parameters was not investigated. Thus, our current study is different from the previous studies.

Different countries have developed maximum permissible limits for AFB1 level in groundnut (Table 1). If groundnut has more than the permissible limit of AFB1, then such groundnut becomes unfit for consumption and trade as well. In Ethiopia, permissible limit for total AFs is 15 ppb which is based on [18]. However, limit for AFB1 is not set. Hence, this study has been taken up to get an idea about AFB1 levels in groundnut germplasm of Ethiopia. The present study is in continuance to our previous study on unravelling the connection of certain parameters such as colonization by *A. flavus* on groundnut seeds, individual seed weight and altitude of cultivation with total free amino acids, total carbohydrate and protein in the groundnut germplasm of Ethiopia [19]. In the present study, the relationship of AFB1 with altitude of cultivation, individual seed weight, kernel colonization and certain biochemical variables are investigated. Thus, this research focused on investigation of AFB1 and factors influencing AFB1 levels in groundnut (*Arachis hypogaea* L) germplasm in selected areas of Ethiopia.

2. Materials and methods

2.1. Sample collection

This is a cross-sectional study that involved the collection of 28 groundnut accessions/landraces from Ethiopian Biodiversity Institute (EBI), Addis Ababa. The EBI gathered the germplasm from traditional farming populations which cultivated these locally adapted, traditional varieties in diverse locations as detailed in Tables 2 and 3, under rain-fed conditions, following the guidelines prescribed by Werer Agricultural Research Center, Afar Region, Ethiopia. Among the 28 accessions, 13 are from Babile woreda in the Misrak Harerge zone of the Oromiya region, 7 are from Gursum woreda in the same zone, also within the Oromiya region, and 8 are from the Benishangul Gumuz region.

Cultivation practices are briefly given as follows. The cultivation started with ploughing the land to fine tilth. Fertilizer was applied at a rate of 121 kg NPS per hectare. Prior to sowing at a depth of approx. 3 cm, seeds underwent treatment with the fungicide Mancozeb at a concentration of 4 g kg^{-1}. Carbaryl 10 % DP was applied in the soil during seeding to combat termites, ants and earwigs. Typically, a spacing of 60 cm between rows and 10 cm within rows was maintained. Weeding was carried out manually. Planting occurred in June/July 2019, with harvesting taking place in September/October 2019. Harvested plants were left in the field for several days to undergo air and sun drying, aiming to reduce pod moisture content to <7 %. The pods were stored in gunny bags (25 kg per bag) and stored in ventilated godowns. Six-month old pods were transported to the laboratory in sterile bags kept in a cold box with icepacks (~6 °C).

2.2. Assay of aflatoxin B1

AFB1 ELISA kit (product code: KA07202H) was procured from Beijing Kwinbon Biotechnology Co., Ltd, China. This product is based on indirect competitive Enzyme Linked Immunosorbent Serologic Assay (ELISA). Assay protocol was performed as per manufacturer's instructions (given as supplementary data). The absorbance values were first converted into absorbance^{-1}. Then, the concentrations of the standard, AFB1 (ppb), were displayed on the Y-axis and the absorbance^{-1} values of the standards were presented on the X-axis.

2.2.1. Sample preparation

For AFB1 analysis, pods were withdrawn from different parts of the gunny bags, i.e., the upper, middle and lower parts that

Table 1
Country-wise maximum permissible limits for aflatoxin B1 levels in groundnut.

Country	Foodstuff	Aflatoxin B1 Limit (ppb)	Reference
China	Groundnut and groundnut products	20	[b,c]
European Union	Groundnut ready to eat	2	[a,b]
	Groundnut for further processing	8	[a,c]
Hong Kong	Groundnut	20	[a]
India	all foods	30	[c]
Indonesia	Groundnut	15	[c]
Israel	Groundnut	5	[b]
Japan	all foods	10	[b]
Mauritius	Groundnut	5	[a]

[a] The Almond Board of California, 2022 [38].
[b] De Oliveira & Corassin, 2014 [39].
[c] Norlia et al., 2019 [40].

Table 2
Groundnut accessions from Oromiya region, Misrak Harerge zone.

Region, Zone	Woreda	Locality	Accession no.	Latitude	Longitude	Altitude (meter)
Oromiya region, Misrak Harerge Zone	Babile	Berkele/Shek Abdi about 4 km from Babile town	19739	09-12-25-N	42-21-26-E	1636
		Awsherithacho about 5 km from Babile town on the way to Fek	19742	09-09-21-N	42-22-21-E	1603
		Awsherithacho about 5 km from Babile town on the way to Fek	19743	09-09-21-N	42-22-21-E	1603
		IfaGendeGure, about 4 km from Babile to Kito	19745	09-14-42-N	42-18-22-E	1666
		IfaGendeTegero, about 6 km from Babile to Kito	19747	09-15-30-N	42-18-21-E	1700
		Medigana Bishan Babile, about 8 km from Babile town to Abdig	19748	09-16-06-N	42-18-12-E	1709
		DendaroAbdiguchi about 10 km from Babile to Gambella	19750	09-17-25-N	42-17-25-E	1744
		GendeUmer about 9 km from Babile town on the way to Gemechu	19754	09-09-37-N	42-18-50-E	1509
		GemechuGende Ahmed Alie about 14 km from Babile town	19755	09-07-25-N	42-18-54-E	1459
		Tula about 3 km from Babile town to Abdul kader (Gemechu)	19757	09-12-17-N	42-19-38-E	1604
		Abdul kaderGeydo about 3 km from Babile town to Gemechu	19758	09-11-49-N	42-19-43-E	1573
		Dadi is located about 35 km south west of Harar town	29572	09-08-57-N	42-14-52-E	1288
		ElemoDara is located about 40 km south west of Harar	29574	09-08-49-N	42-13-42-E	1350
	Gursum	IlalemiGendeZeyad, about 5 km from Fuganbirra town	19760	09-19-33-N	42-26-05-E	1898
		IlalemiGendeZeyad, about 5 km from Fuganbirra town	19761	09-19-33-N	42-26-05-E	1898
		Oda Oromia GendeDaroto about 5 km from Fuganbirra town	19765	09-18-40-N	42-28-38-E	1765
		Oda Oromia GendeHindebra about km from Fuganbirra town	19766	09-18-30-N	42-29-41-E	1753
		NurSelam about 12 km from Fuganbirra km from the junction	19768	09-19-30-N	42-28-29-E	1651
		NurSelam about 12 km from Fuganbirra km from the junction	19769	09-19-30-N	42-28-29-E	1651
		OdaaSantalaGendeKuri about 12 km from Fugdabirra town	19771	09-21-54-N	42-29-50-E	1607

Table 3
Groundnut accessions from Benishangul & Gumuz region.

Region, Zone	Woreda	Locality	Accession no.	Latitude	Longitude	Altitude (meter)
Benishangul Gumuz region, Metekel Zone	Guba	Babezendakebele from Guba to Babezendakebele 30 km	23521	11-08-00-N	35-28-09-E	614
		Bakambel from Guba to besbata 21 km	23524	11-06-81-N	35-25-60-E	601
		Megenteya Got from Guba to megnteja got 9 km	23525	11-16-28-N	35-22-12-E	837
		Mankushzuria from Guba to Mankushzurya 4 km	23528	11-16-02-N	35-19-30-E	850
		Mankushzuria from Guba to Mankushzurya 4 km	23529	11-16-18-N	35-21-39-E	808
	Bulen	Tachmara from Bulen to Tume 16 km	23531	10-31-47-N	36-01-10-E	1480
	Assosa	Got 9 from Asosa to Amba 9 Got to 15 km	23532	10-00-35-N	34-36-34-E	1507
Benishangul Gumuz region, Kamash Zone	Blo Jiganifado	Say Deacha	29488	09-12-32-N	36-12-43-E	1227

weighed *in toto* 3 kg. Then, the pods were shelled, mixed together and powdered using a kitchen blender aseptically. In a 50 ml polystyrene centrifuge tube, 2 g of seed powder was taken. To this, initially 3 ml of acetonitrile was added, followed by the addition of 3 ml of deionized water. The mixture was vortexed for 5 min and then centrifuged at 3000 *g* for another 5 min at room temperature. Subsequently, 3 ml of the resulting supernatant was transferred to another 50 ml polystyrene centrifuge tube. To this, 4.5 ml of chloroform was added, followed by another round of vortexing for 5 min and centrifugation at 3000*g* for 5 min at room temperature. Next, 3 ml of the substrate organic phase was transferred to a clean 10 ml glass tube and dried under a flow of nitrogen gas at 50–60 °C. Once dry, 1 ml of *n*-hexane was added, and the mixture was shaken for 30 s. Following this, 1 ml of the extraction solution provided by the kit manufacturer was added, and the mixture was shaken again for 30 s. The tube was then centrifuged at 3000 *g* for 5 min at room temperature, after which the supernatant organic phase was discarded. Finally, 50 μl of the substrate water phase was retrieved for the assay. This procedure was repeated thrice to get triplicate data.

2.3. Other parameters

Variables namely kernel colonization by *Aspergillus flavus*, individual seed weight, total carbohydrates, protein and total free amino acids were estimated by us earlier [19].

2.4. Statistical analysis

As previously done by Hamidou et al. [20], logarithmic (base 10) conversions of AFB1 concentration (ppb) were utilized to stabilize the variance as the data for AFB1 exhibited a diverse range of values. Critical difference (CD) values were estimated at 1 % and 5 % levels using WASP 2.0 (https://ccari.icar.gov.in/waspnew.html) to see if there are any significant differences among the accessions. Relationship of AFB1 with other studied parameters was determined by computing the correlation coefficient (r) values.

3. Results and discussion

AFB1 was determined in 28 groundnut accessions in triplicates by using indirect competitive ELISA. The standard graph used for the calculation AFB1 in the groundnut samples is presented in Fig. 1. Trend line is obtained using polynomial option with order 3 in Microsoft® Excel® 2016. The trend line in this graph was obtained using polynomial option with order 3 in Microsoft® Excel® 2016. The AFB1 levels in ppb ranged from the lowest value of 1.94 in the accessions 19750 & 23521 to the highest value of 410.7 in the accession 19742. These findings are in agreement with previous studies on aflatoxin contamination in Ethiopian groundnut. Bisrat & Gebre [15] reported an average AFB1 level of 34.7 ppb. Amare et al. [14] found aflatoxin levels ranging from 5 to 250 ppb in eastern Ethiopia. Chala et al. [16] analyzed groundnut samples from farmers' stores and markets and found significant contamination. Over 77 % of their samples tested positive for aflatoxin, with levels varying widely from 15 to a concerning 11,900 ppb. A study by Mohammed et al. [17] investigated groundnut samples collected from farmers' stores in Eastern Ethiopia. They found that a significant portion of the samples from Babile district were contaminated with AFB1. In the 2013/14 cropping season, 45 % of the samples tested positive for AFB1, with levels ranging from 1.8 to 723 ppb. This contamination rate decreased to 30 % in the 2014/15 season, but the levels of AFB1 were higher, ranging from 7.1 to 2526 ppb. It is imperative to note here that all the 28 groundnut accessions investigated in the present study tested positive for AFB1. No level of aflatoxin above zero is considered safe [21].

Studies from around the globe reveal concerning levels of aflatoxin contamination in groundnuts. In Thailand, Arunyanark et al.

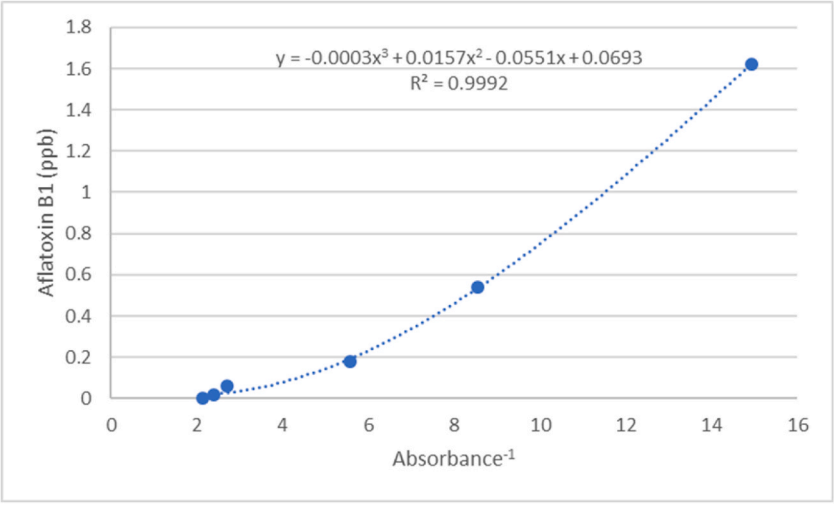


Fig. 1. Standard graph used for AFB1 measurement.

[22] found significant variation among groundnut varieties, with aflatoxin levels ranging from a low of 4 ppb to a high of 183 ppb. Monyo et al. [23] reported AFB1 levels as high as 3240 ppb in fresh groundnut samples from Malawi. Waliyar et al. [24] documented AFB1 in groundnut samples from Malian markets, ranging from 105 to 226.3 ppb. Kachapulula et al. [25] analyzed groundnuts in Zambia and found an average total aflatoxin content of 39 ppb. Muzoora et al. [26] detected total aflatoxin levels ranging from 1.6 to 516 ppb in Ugandan groundnut samples collected from traders. Oyedele et al. [27] found that nearly 30 % of domestic market groundnut samples in Nigeria tested positive for AFB1, with levels ranging from 0.9 to 710 ppb. In Ghana, Kortei et al. [21] reported that over 61 % of market samples contained AFB1, ranging from 0.38 to 230.21 ppb. A recent study by Kimario et al. [28] found an alarming average total aflatoxin content of 269 ppb in household-stored groundnut samples from Dodoma, Tanzania. Aflatoxin contamination in groundnuts appears to be a widespread problem across Africa and also in other continents. The severity of the contamination varies depending on the location. Further research is needed to understand the underlying causes of aflatoxin contamination variations and develop effective control strategies in different regions.

The standard deviation in AFB1 content in the current study was 132.58. Because a wide range of values of AFB1 (ppb) was found, logarithmic (base 10) transformations of AFB1 (ppb) were used to stabilize the variance in the current investigation. Log_{10} transformation resulted in almost equal correlation coefficient (r) values for transformed and untransformed data of EBI accessions. *i.e.*, the r value between untransformed data [AFB1 (ppb)] and kernel colonization was 0.855 ($p < 0.01$), while the r value between transformed data [log_{10} AFB1] and kernel colonization was 0.873 ($p < 0.01$). Similarly, the r value between untransformed data [AFB1 (ppb)] and total carbohydrates was 0.620 ($p < 0.01$), while the r value between transformed data [log_{10} AFB1] and total carbohydrate was 0.573 ($p < 0.01$). Log_{10} transformation of AFB1 is necessary to compare all the accessions using a histogram. The standard deviation has reduced to 0.85 due to log_{10} transformation. Therefore, log transformed AFB1 values are discussed in the present investigation.

Log_{10} AFB1 content in the groundnut landraces belonging to Oromiya region, Misrak Harerge zone is presented in Fig. 2. The European Union has established a threshold limit for AFB1 at 2 ppb in ready to eat groundnut. Log_{10} of 2 ppb is 0.30. Hence, accessions showing log_{10} AFB1 values > 0.30 are unacceptable in European Union. The present study reveals that the accessions namely, 19750, 19754 and 29574 are only acceptable in European Union. The remaining 17 accessions in are unacceptable due to high AFB1 content. Log_{10} AFB1 content in the groundnut accessions belonging to Benishangul Gumuz region is presented in Fig. 3. The accessions namely, 23521, 23524, and 23525 are acceptable in European Union, while the remaining five accessions are unacceptable as they contain >0.30 log_{10} AFB1.

An insignificant relationship ($r = 0.246$) was noticed between altitude of cultivation and log_{10} AFB1 in the present study. Guchi

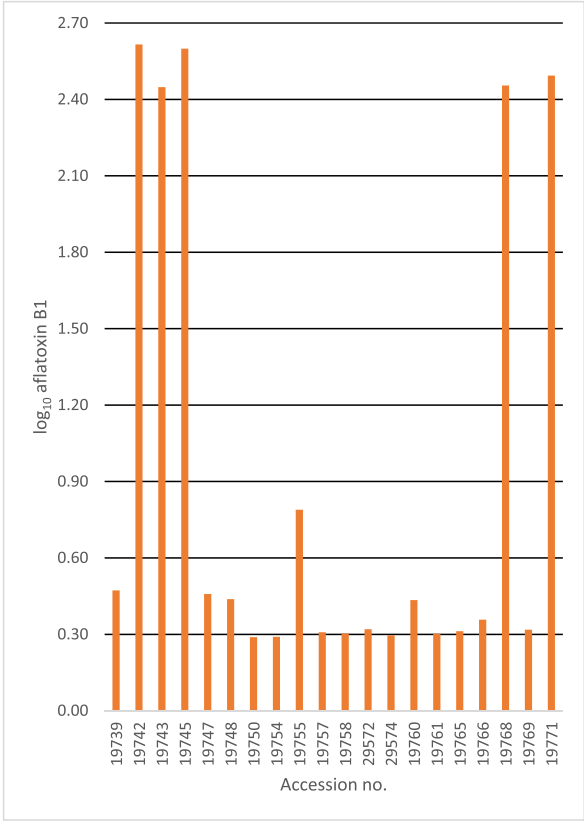


Fig. 2. Log_{10} AFB1 content in the groundnut accessions from Oromiya region, Misrak Harerge zone.

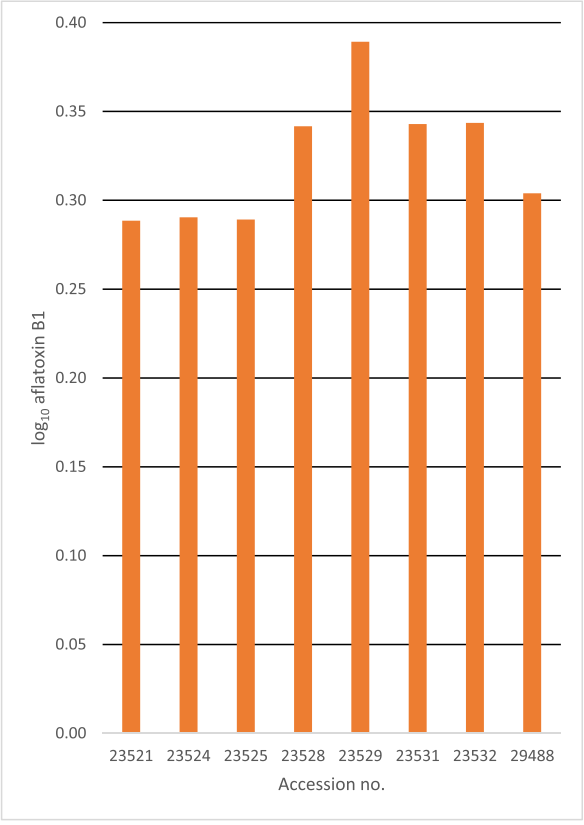


Fig. 3. Log_{10} AFB1 content in the groundnut accessions from Benishangul Gumuz region.

[29] observed that aflatoxin levels are greatly affected by environmental conditions. He [29] further added that the danger of aflatoxin contamination rises in grain crops after an increase in temperature. Species that produce aflatoxin necessitate temperatures ranging from 25 to 37 °C and a moisture level between 80 % and 85 % for their growth [30]. In Malawi, aflatoxin contamination typically shows greater prevalence in the hotter, lower altitudes along the lakeshore of Lake Malawi and the Shire Valley, in contrast to the cooler, higher altitudes of Mchinji, Kasungu, and the Lilongwe plateau [23]. The study by Monyo et al. [23] showed that the hotter regions of Malawi exhibited elevated populations of aflatoxigenic fungi. This observation is contrary to that in Zambia, where Njoroge et al. [31] noted that populations of aflatoxigenic fungi were higher in the cooler plateau regions in contrast to those in the hot Luangwa Valley. It is concluded based on the data of the present study that altitude of cultivation has no effect on AFB1 content in groundnut accessions of Ethiopia.

Table 4 presents the data on individual seed weight, kernel colonization by *A. flavus*, total carbohydrates, protein, total free amino acids, and log_{10} AFB1 in Ethiopian groundnut germplasm. Statistical analysis showed that there are significant variations ($P < 0.05$; $P < 0.01$) among the accessions in terms of all the investigated variables. Kernel colonization exhibited a positive relationship with log_{10} AFB1, which is significant at 1 % level (Table 5). This is because kernel colonization by *A. flavus* in the initial stage results in AFB1 accumulation in the final stage. In the studies conducted by Arunyanark et al. [22], under the irrigated treatment, there was a decrease in kernel colonization (2–37 %) and aflatoxin contamination [1–19ppb], in contrast to severe drought conditions where kernel colonization could reach up to 53 % and aflatoxin contamination up to 62 ppb. The kernel colonization and aflatoxin contamination were consistently lower in the genotypes ICGVs 98324, 98330, and 98353 [22].

A significant ($p < 0.01$) positive correlation between Log_{10} AFB1 content and total carbohydrates was also observed in the present study (Table 5). This indicates that carbohydrate content in the groundnut seeds has relationship with AFB1 content. Basic sugars like glucose, sucrose, and fructose, which serve as the main soluble sugars in groundnuts, have been noted as effective carbon reservoirs for aflatoxin production in controlled laboratory settings [32]. In times of drought and temperature strain, the carbohydrate levels in groundnut seeds experience an elevation [33]. Moreover, immature seeds typically contain higher levels of sugar compared to mature seeds, and it is more common for aflatoxin contamination to occur in these immature seeds [34]. Noted variances in carbohydrate content hold significance because Manda et al. [34] reported strong positive associations between aflatoxin levels in groundnuts and the sucrose, fructose, and glucose content present in the groundnuts at harvest. The carbohydrate levels in developing groundnut seeds or those subjected to drought stress might have a pivotal influence on aflatoxin production [32].

Table 4
Certain parameters analyzed in the groundnut accessions of Ethiopia.

S. No.	Access-ion no.	Individual seed weight (g)	Kernel coloni-zation (%)	Total carbohydr-ates (mg/100 mg)	Protein (mg/ 100 mg)	Total free amino acids (mg/100 mg)	log_{10} AFB1
1	19739	0.519	20	15.47	26.86	3.76	0.474 (2.98)
2	19742	0.525	100	21.05	23.69	4.11	2.614 (410.70)
3	19743	0.585	90	11.33	21.21	3.96	2.448 (280.81)
4	19745	0.621	100	21.89	21.49	4.2	2.597 (395.15)
5	19747	0.666	30	10.78	26.02	3.64	0.458 (2.87)
6	19748	0.663	10	12.9	21.78	5.85	0.439 (2.75)
7	19750	0.66	0	12.18	23.15	6.89	0.289 (1.94)
8	19754	0.623	0	8.44	29.2	1.34	0.290 (1.95)
9	19755	0.626	60	10.05	23.89	3.78	0.789 (6.15)
10	19757	0.599	40	14.3	17.09	3.48	0.308 (2.03)
11	19758	0.516	10	14.45	16.79	3.7	0.303 (2.01)
12	29572	0.417	10	13.56	28.95	0.47	0.320 (2.09)
13	29574	0.522	10	12.08	29.98	0.82	0.296 (1.98)
14	19760	0.553	0	17.49	25.76	2.42	0.435 (2.72)
15	19761	0.528	10	11.78	20.38	2.75	0.302 (2.01)
16	19765	0.505	50	11.89	23.57	1.37	0.312 (2.05)
17	19766	0.571	50	17.41	29.9	1.28	0.358 (2.28)
18	19768	0.606	70	17.16	39.49	1.2	2.455 (285.26)
19	19769	0.472	10	14.6	32.52	1.43	0.318 (2.08)
20	19771	0.522	70	14.83	23.95	0.37	2.494 (311.98)
21	23521	0.788	0	7.02	31.76	1.64	0.289 (1.94)
22	23524	0.738	0	8.64	24.91	2.04	0.290 (1.95)
23	23525	0.776	0	9.94	26.33	2.29	0.289 (1.95)
24	23528	0.644	0	8.32	22.53	2.27	0.342 (2.20)
25	23529	0.697	0	10.64	25.45	1.9	0.389 (2.45)
26	23531	0.788	0	10.84	22.47	1.03	0.343 (2.20)
27	23532	0.457	10	13.3	21.44	0.51	0.344 (2.21)
28	29488	0.531	0	11.13	22.17	0.76	0.304 (2.01)
CD (0.05)		0.032	4.60	6.14	5.67	0.899	0.025
CD (0.01)		0.043	6.10	8.18	7.55	1.197	0.033
CV		3.33	12.22	28.93	13.81	22.234	2.031

Values in parenthesis are the ppb value of AFB1.

Table 5
Linear relationship of log_{10} AFB1 with total carbohydrates (mg/100 mg) and kernel colonization (%), using the data obtained from EBI groundnut accessions. Regression formula $Y = a + b X$, where X is log_{10} AFB1, a and b are regression constants.

Y	Correlation coefficient (r)	a	b	n
Total carbohydrate (mg/100 mg)	0.573[a]	11.147	2.459	28
Kernel colonization (%)	0.873[a]	1.21	34.283	28

n is number of observations.
[a] $p < 0.01$.

4. Conclusions

AFB1 level and its relationship with various parameters have been studied in the groundnut germplasm of Ethiopia. The AFB1 levels measured by indirect competitive ELISA ranged from 1.94 ppb to 410.7 ppb. Out of the 28 accessions studied, only six accessions contained ≤2 ppb AFB1 and so, they comply with maximum permissible limit set by European Union. It was found out that altitude do not influence AFB1 content in groundnut. Log_{10} AFB1 exhibited a positive relationship ($p < 0.01$) with kernel colonization, and also with total carbohydrates. It is put forward that an increase in total carbohydrate favours initial kernel colonization by *A. flavus* and final AFB1 accumulation in groundnut kernels. AFB1 measurement is an expensive technique. Hence, kernel colonization has been recommended as an inexpensive analytical tool to get an idea about AFB1 levels in groundnut. Farmers are recommended to cultivate the groundnut genotypes that are reported to contain low levels of AFB1. For the groundnut genotypes which exceed maximum permissible limit for AFB1, appropriate pre- and post-harvest aflatoxin management practices should be developed. Besides, the data generated in this study can be used by the government to fix maximum permissible limit for AFB1 in the groundnut seeds of Ethiopia. It is imperative to note here that the data generated in the present study especially AFB1 content could change in the subsequent years due to climate change.

As aflatoxin contamination in groundnut is greatly influenced by genotype - environment interaction, breeding for aflatoxin resistance is cumbersome. There is not one route for the fungus to reach the kernel. For example, the kernel may be exposed to the fungi before harvest because of lack of plant genetic resistance or after harvest due to improper drying, transportation and poor storage facilities. Many methods have been used to mitigate aflatoxin levels such as late season irrigation, biocontrol using atoxigenic *A. flavus*, weed & insect control, timely planting & harvesting, and habitat management employing push-pull technology [35]. Recently, Kortei et al. [36] were able to achieve 97.38 % reduction of aflatoxins by means of parboiling groundnut seeds in brine solution before drying and roasting with sand. Guo et al. [37] found out that the AFB1 levels in groundnut seeds could be brought down to undetectable levels even after 60 days of storage if the soil used for cultivation was fumigated using chloropicrin or metham sodium. So as of now, it is not possible to prevent aflatoxin contamination using a single strategy. The use of a combination of different methods based on the available resources in a locality is the only option now to prevent aflatoxin contamination in groundnut.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on reasonable request.

CRediT authorship contribution statement

Yonas Syraji: Methodology, Investigation. **Jeyaramraja PR:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **Teklu Wegayehu:** Supervision, Project administration, Investigation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used chatGPT in few places of the manuscript, in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35023.

References

[1] G. Kudama, Economics of groundnut production in east hararghe zone of oromia regional state, Ethiopia, Sci. Technol. Arts Res. J. 2 (2) (2013 Dec 17) 135–139.
[2] M.H. Ahmed, H.M. Mesfin, S. Abady, W. Mesfin, A. Kebede, Adoption of improved groundnut seed and its impact on rural households' welfare in Eastern Ethiopia, in: G. Aye (Ed.), Cogent Economics & Finance 4 (1) (2016 Dec 31) 1268747.
[3] M.K. Pandey, R. Kumar, A.K. Pandey, P. Soni, S.S. Gangurde, H.K. Sudini, et al., Mitigating aflatoxin contamination in groundnut through A combination of genetic resistance and post-harvest management practices, Toxins 11 (6) (2019 Jun 3) 315.
[4] R.G. Popescu, A.L. Rădulescu, S.E. Georgescu, A. Dinischiotu, Aflatoxins in feed: types, metabolism, health consequences in swine and mitigation strategies, Toxins 14 (12) (2022 Dec 3) 853.
[5] G.A. Payne, N.W. Widstrom, Aflatoxin in maize, Crit. Rev. Plant Sci. 10 (5) (1992 Jan) 423–440.
[6] Aflatoxins Benkerroum, Producing-molds, structure, health issues and incidence in southeast asian and sub-saharan African countries, IJERPH 17 (4) (2020 Feb 13) 1215.
[7] P.R. Jeyaramraja, S. Nithya Meenakshi, F. Woldesenbet, Relationship between drought and preharvest aflatoxin contamination in groundnut (Arachis hypogaea L.), World Mycotoxin J. 11 (2) (2018 Feb 7) 187–199.
[8] G.S. Shephard, Aflatoxin analysis at the beginning of the twenty-first century, Anal. Bioanal. Chem. 395 (5) (2009 Nov) 1215–1224.
[9] M. Atongbiik Achaglinkame, N. Opoku, F.K. Amagloh, Aflatoxin contamination in cereals and legumes to reconsider usage as complementary food ingredients for Ghanaian infants: a review, Journal of Nutrition & Intermediary Metabolism 10 (2017 Dec) 1–7.
[10] G.S. Bbosa, D. Kitya, A. Lubega, J. Ogwal-Okeng, W.W. Anokbonggo, D.B. Kyegombe, Review of biological and health effects of aflatoxins on body organs and body systems, in: Aflatoxins-recent Advances and Future Prospects, Intech, 2013, pp. 239–269.
[11] N.K. Kortei, R.A. Tetteh, M. Wiafe-Kwagyan, D.N.K. Amon, G.T. Odamtten, Mycobiota profile, phenology, and potential toxicogenic and pathogenic species associated with stored groundnuts (Arachis hypogaea L.) from the Volta Region, Ghana, Food Sci. Nutr. 10 (3) (2022 Mar) 888–902.
[12] J.P. Meneely, O. Kolawole, S.A. Haughey, S.J. Miller, R. Krska, C.T. Elliott, The challenge of global aflatoxins legislation with a focus on peanuts and peanut products: a systematic review, Expo Health 15 (2) (2023 Jun) 467–487.
[13] E. Guchi, Aflatoxin contamination in groundnut (Arachis hypogaea L.) caused by Aspergillus species in Ethiopia, Journal of Applied & Environmental Microbiology 3 (1) (2015) 11–19.

[14] A. Amare, A. Dawit, H. Mengistu, Mycoflora, aflatoxins and resistance of groundnut cultivars' from Eastern Ethiopia, Sinet 18 (1995) 117–131.
[15] A. Bisrat, P. Gebre, A preliminary study on the aflatoxin content of selected Ethiopian food, Ethiop. Med. J. 19 (1981) 47–52.
[16] A. Chala, A. Mohammed, A. Ayalew, H. Skinnes, Natural occurrence of aflatoxins in groundnut (Arachis hypogaea L.) from eastern Ethiopia, Food Control 30 (2) (2013 Apr) 602–605.
[17] A. Mohammed, A. Chala, M. Dejene, C. Fininsa, D.A. Hoisington, V.S. Sobolev, et al., Aspergillus and aflatoxin in groundnut (Arachis hypogaea L.) and groundnut cake in Eastern Ethiopia, Food Addit. Contam. B 9 (4) (2016 Oct) 290–298.
[18] GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED, CXS 193-1995, CODEX ALIMENTARIUS, Food and Agriculture Organization of the United Nations; World Health Organization, 2019. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url. (Accessed 3 November 2023).
[19] S. Yonas, P.R. Jeyaramraja, Relationship of altitude, individual seed weight, and kernel colonization by Aspergillus flavus with biochemical parameters of various Ethiopian groundnut (Arachis hypogaea L.) accessions, J Exp Bio & Ag Sci. 10 (2) (2022 Apr 30) 344–358.
[20] F. Hamidou, A. Rathore, F. Waliyar, V. Vadez, Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination, Field Crops Res. 156 (2014 Feb) 103–110.
[21] N.K. Kortei, T. Annan, P.T. Akonor, S.A. Richard, H.A. Annan, M.W. Kwagyan, et al., Aflatoxins in randomly selected groundnuts (Arachis hypogaea) and its products from some local markets across Ghana: human risk assessment and monitoring, Toxicol Rep 8 (2021) 186–195.
[22] A. Arunyanark, S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot, G.C. Wright, et al., Association between aflatoxin contamination and drought tolerance traits in peanut, Field Crops Res. 114 (1) (2009 Oct) 14–22.
[23] E.S. Monyo, S.M.C. Njoroge, R. Coe, M. Osiru, F. Madinda, F. Waliyar, et al., Occurrence and distribution of aflatoxin contamination in groundnuts (Arachis hypogaea L) and population density of Aflatoxigenic Aspergilli in Malawi, Crop Protect. 42 (2012 Dec) 149–155.
[24] F. Waliyar, M. Osiru, B.R. Ntare, K.V.K. Kumar, H. Sudini, A. Traore, et al., Post-harvest management of aflatoxin contamination in groundnut, Wis. Med. J. 8 (2) (2015 Jan 1) 245–252.
[25] P.W. Kachapulula, J. Akello, R. Bandyopadhyay, P.J. Cotty, Aflatoxin contamination of groundnut and maize in Zambia: observed and potential concentrations, J. Appl. Microbiol. 122 (6) (2017 Jun) 1471–1482.
[26] S. Muzoora, M.L. Khaitsa, H. Bailey, P. Vuzi, Status on aflatoxin levels in groundnuts in Uganda, PAMJ 27 (4) (2017) 11.
[27] O.A. Oyedele, C.N. Ezekiel, M. Sulyok, M.C. Adetunji, B. Warth, O.O. Atanda, et al., Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria, Int. J. Food Microbiol. 251 (2017 Jun) 24–32.
[28] M.E. Kimario, A.P. Moshi, H.P. Ndossi, J.B. Kussaga, Aflatoxin contamination of household stored grains for smallholder farmers in Dodoma, Tanzania, World Mycotoxin J. 17 (1) (2024 Feb 1) 73–84.
[29] E. Guchi, Implication of aflatoxin contamination in agricultural products, American Journal of Food and Nutrition 3 (1) (2015) 12–20.
[30] P. Battilani, P. Toscano, H.J. Van Der Fels-Klerx, A. Moretti, M. Camardo Leggieri, C. Brera, et al., Aflatoxin B1 contamination in maize in Europe increases due to climate change, Sci. Rep. 6 (1) (2016 Apr 12) 24328.
[31] S.M.C. Njoroge, K. Kanenga, M. Siambi, F. Waliyar, E.S. Monyo, Identification and toxigenicity of Aspergillus spp. from soils planted to peanuts in eastern Zambia, Peanut Sci. 43 (2) (2016 Jul 1) 148–156.
[32] S.S. Uppala, K.L. Bowen, F.M. Woods, Pre-harvest aflatoxin contamination and soluble sugars of peanut, Peanut Sci. 40 (1) (2013 Jan 1) 40–51.
[33] M.N. Musingo, S.M. Basha, T.H. Sanders, R.J. Cole, P.D. Blankenship, Effect of drought and temperature stress on peanut (Arachis hypogaea L.) seed composition, J. Plant Physiol. 134 (6) (1989 Aug) 710–715.
[34] A. Manda, B. Naidu, C. NageswaraRao, Graeme W. Rachaputi, F. Shu, Aflatoxins and their relationship with sugars in peanut (Arachis hypogaea L.) (2004). Brisbane, Australia, http://www.cropscience.org.au/icsc2004/poster/5/1/3/625_manda.htm#TopOfPage. (Accessed 11 February 2024).
[35] N.K. Njeru, C.A.O. Midega, J.W. Muthomi, J.M. Wagacha, Z.R. Khan, In vitro antifungal activity of Desmodium intortum and D. uncinatum root extracts against growth of toxigenic Fusarium verticillioides and Aspergillus flavus, Aust. J. Crop. Sci. 14 (12) (2020 Dec 10) 1942–1948, 2020.
[36] N.K. Kortei, S. Osei, A.A. Addae, P.T. Akonor, N.Y.B. Manaphraim, T. Annan, Impact of some Ghanaian local roasting methods on aflatoxin reduction in groundnuts (Arachis hypogaea L), Scientific African 23 (2024 Mar) e02022.
[37] A. Guo, Y. Zhang, Y. Ji, X. Chen, W. Zhang, X. Liu, et al., The potential for reducing aflatoxin B1 contamination of stored peanuts by soil disinfection, J. Hazard Mater. 469 (2024 May) 133916.
[38] The Almond Board of California, International Aflatoxin Tolerances, 2022 Jan. California. (Accessed 3 November 2023).
[39] C.A. De Oliveira, C.H. Corassin, Aflatoxins, in: Mycotoxins and Their Implications in Food Safety [Internet]. Unitec House, 2 Albert Place, London N3 1QB, UK, Future Science Ltd, 2014, pp. 3–15. https://www.futuremedicine.com/doi/10.4155/ebo.13.468. (Accessed 3 November 2023).
[40] M. Norlia, S. Jinap, M.A.R. Nor-Khaizura, S. Radu, N.I.P. Samsudin, F.A. Azri, Aspergillus section flavi and aflatoxins: occurrence, detection, and identification in raw peanuts and peanut-based products along the supply chain, Front. Microbiol. 10 (2019 Nov 22) 2602.