Aflatoxin Contamination Association with Seed Coat Biochemical Markers in Peanut under Intermittent Drought

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Article

Aflatoxin Contamination Association with Seed Coat Biochemical Markers in Peanut under Intermittent Drought

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Abstract: Aflatoxin contamination (AC) increases as the severity of drought stress increase in peanut. Identifying drought-tolerant (DT) genotypes with resistance to *Aspergillus. flavus* in semi-arid tropics may aid in development of peanuts that minimizing aflatoxin contamination. The goal is to identify the DT genotypes and resistant to aflatoxin contamination. The experiments curried out at ICRISAT Sahelian Center on fifty-five genotypes assessed in adjacent intermittent water-stressed (WS) from 60th day after sowing to maturity and well-watered (WW) conditions in a randomized complete block design. The yields and components, incidence of *A. flavus* colonisation, aflatoxin contamination and the seed coat total pthe olyphenol (SCTPP) were estimated. Water deficit reduced pod yield, seed yield, and haulm yield up to 19.49%, 27.24% and 22.07% respectively and increased the immature pods plant^{-1}, (IMPN) and aflatoxin contamination up to 67.16% and 54.95%. The genotypes ICG 2106, ICG 311, ICG 4684, ICG 4543, and ICG 1415 maintained high yield under WS, less of IMPN and the lowest AC variation between WW and WS. The drought tolerant genotypes showed the relationship with aflatoxin resistance and SCTPP (r2=0.80; r2=0.82) under WW and WS. These findings can be used to select the genotype that combine drought tolerance and minimizing aflatoxin contamination.

Keywords: Peanut; intermittent drought; seed coat; aflatoxin

1. Introduction

Peanut (*Arachis hypogaea* L) is the second important cash crop in Niger which is produced in five of eight regions of the country. It is called 'women crop' because extensive involvement of women in peanut production and processing. Peanut pods grow underground, their development is directly influenced by the water conditions of the surrounding soil [1]. As an underground crop, the pods are subjected to continuous risk of direct contact with populations of aflatoxigenic aspergilli in the soil [2]. Aflatoxin contamination is the most important quality problem in peanuts throughout the world as it is related to serious health problems in human as well as in livestock [3].

Peanut grown in the Sahel often experiences water deficits during the pod-filling phase, which usually coincides with the end of the rainy season [4]. In this zone, peanut production is often affected by an intermittent drought which is an episodic water deficit during plant growth [5]. Previous studies in Niger demonstrated that drought stress for less than ten days was enough to cause significant aflatoxin contamination in the field [6,7]. However, increased duration of terminal drought and temperature are major factors determining the level of aflatoxin contamination [6].

Precisely, at the pod filling stage, drought cause plant stress and produce lower phytoalexin [8] then *A. flavus* can colonize in peanut pods prior to harvest and the contamination of aflatoxin is more severe under terminal drought [9,10]. Indeed, drought can cause cracking of seed coats, which in turn

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permits ingress of *A. flavus* germinating spores and hyphae into embryonic tissues [11]. Recent studies observed that varieties subjected to drought during late growth stages had different levels of resistance to aflatoxin contamination [9]. The different levels of resistance would be linked firstly to drought tolerance mechanisms, either by escape, tolerance or avoidance, may impact the ability of genotypes to minimize aflatoxin production by maintaining kernel water activities allowing phytoalexin production [12]. Secondly, the seed coats and outer shells play vital roles in protecting the seeds from mechanical damage, pest infestation and surviving harsh weather conditions [13]. Then, a combined approach of host resistance followed by pre- and post-harvest management practices is required [14].

Aflatoxin contamination can be minimized by adopting certain cultural, produce handling, and storage practices [12]. However, these practices are not widely adopted particularly by the small farmers in the developing countries, which contribute about 60% to the world peanut production. One of the possible means of reducing aflatoxin contamination of peanut is the use of cultivars resistant to seed invasion by aflatoxin producing fungi or to aflatoxin production. This suggests that drought tolerant genotypes may possess some degree of tolerance to aflatoxin contamination and it has been argued that drought tolerance traits in peanut may have the potential to be used as indirect selection criteria for resistance to pre-harvest aflatoxin contamination [15]. Recent study [16] reported a wide variation between genotypes in incidence, severity, and aflatoxin rate can be biochemical compounds' diff--erential variability in the tested seeds. It was observed the differences in mycelial growth surface coverage were probably attributed to differences in physical and chemical features of the seed-coat, pod-shell thickness and reticulation [17]. The seed coats and outer shells play vital roles in protecting the seeds from mechanical damage, pest infestation and surviving harsh weather conditions [13]. We have recently demonstrated that seed coat acts as a physical and biochemical barrier against *A. flavus* infection [18,19]. To develop peanut cultivars resistant to *A. flavus* infection with reduced aflatoxin contamination, there is a possible defence mechanism at three stages: prevention of fungal invasion in the pericarp, reduction of aflatoxin production the seed coat and cotyledons [20]. Using cultivars resistant to kernel infection by *A. flavus* is also one of the promising ways to reduce aflatoxin contamination. The objective of this study was to identify the drought tolerant and seed coat biochemical traits to *A. flavus* resistance and aflatoxin contamination. The specific objectives are to (1) evaluate peanut genotypes under intermittent water deficit to identify the drought tolerants(2) identify specific biochemical traits associated to *A. flavus* resistance in seed coat and (3) identify the intermittent drought tolerant genotypes with seed coat resistance to *A. flavus* infection and Aflatoxin contamination.

Materials and Methods

2.1. Plant Materials, Temperature and Relative Humidity

Fifty five lines from ICRISAT peanut mini core collection, contrasting for aflatoxin content [21] were selected and seeds were provided by ICRISAT Niger gene bank. The cultivars included as check, 55-437 and J11 are considered as resistant to pre-harvest aflatoxin contamination while JL24 and Fleur11 are susceptible [22]. Two experiments were conducted (August to December) in year1 and year2 under field conditions at the International Crop Research Institute for the Semi-Arid Tropics, (ICRISAT) Sahelian Centre (ISC) in Sadoré (45km south of Niamey, Niger, 13°N, 2°E). During the crop growing period, the maximum (Max) and minimum (Min) air temperatures and the relative humidity (RH) were recorded daily from a meteorological station located close to the experimental field. The Max and Min temperatures varied from 26.4°C to 41.2°C in year1 and from 29.01°C to 39.30°C in year1 while the RH varied from 29.9% to 68.6% in year2 and from 40.0% to 62.23% in year2. The total water received from rainfall and irrigation were 590 mm^3 and 470 mm^3 respectively in were well-watered (WW) and intermittent water-stressed (WS) in year1 and 645 mm^3 and 505 mm^3 in year2.

2.2. Experimental Design and Water Treatment

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The experimental design was alpha lattice with two factors, water treatment as main factor, and genotypes (55) as sub-factor randomized in each water treatment with four replications. The spacing between water treatments was 6 m and 2 m between replications. Within each repetition, there were 55 elementary plots with an area of 2 m x 1 m = 2 m^2 spaced 1 m apart. The plots subject to WS are suitably watered (30 mm^3 per irrigation) like those of the WW treatment until the 60th day after sowing (seed filling stage start) when the water stress was imposed. The WS treatment consisted of stopping irrigation of WS plants until the majority of stressed plants showed clear wilting symptoms before watering and then stopping irrigation again. This cycle continues until the pod's maturity.

2.3. Crop Management

In each experiment, plot area measured 2 m^2 (2m x 1m) including two rows of 2 m length. Rows spacing was 0.5 m and each row had ten hills. Three seeds were sown by hand in each hill, in 3 cm deep after receiving an irrigation of 30 mm using a Linear-move irrigation system (Valmont Irrigation Inc., Valley, Nebraska, USA). Two and three weeks after sowing, the plants were thinned to two and one plants per hill respectively. The plots were subsequently fertilized with 150 kg ha^{-1} N-P_2O_5-K_2O and irrigated with 30 mm of water. The fields were kept free from weeds by manual cultivation, and regular spraying of decis (deltaméthrin 12 CE), Emacot 050WG (Emamectine benzoate 50g/kg) and Benji controlled insect pests.

2.4. Measurements and Data Collection

From the date of sowing days to flowering was recorded when 50% of plants in each plot reached flowers, day to beginning seeds filling stage and 50% seeds filling stage was monitoring to applied water stress to harvest were recorded when 50% of plants in each plot showed mature pods.

Yield components

After harvesting by hand, the plants of each plot (1m^2), the pods number, the immature pods number, the seeds number, the haulm yield, the pods yield, the seeds yield were determined.

2.5. Assessment of Phenotypic Variation to A. Flavus Colonization

The In-Vitro Seed Colonisation (IVSC) was carried out in the laboratory using ten, fifteen, and twenty-five seeds per petri dish. For each genotype, the seeds with seed coat showing no apparent damage were selected. Each petri dish was considered as a replications using a modified method [23]. The *A. flavus* strains was collected from the sorghum seeds exposed at the Laboratory conditions (25°C-28°C) during seven days and multiplication on Potato Dextrose Agar (PDA) for inoculum preparation [23]. The *A. flavus* colonies were collected based on morphological characteristics and homogenized by successive culture on PDA medium. The *A. flavus* conidial suspension was prepared with the spores collected by culture escaping before dilution spores concentration with distilled water and adjusted approximately to 1.9.1×^{6} spores/millilitre (ml) using Haemocytometer [24]. 500 µl of spore suspension was inoculated in each petri dish before incubation at 28°C for seven days in darkroom. The observation was recorded for percent of colonization after the incubation. Individual seeds were scored for surface colonisation by *A. flavus* and for colonization severity using the following rating scale. 1=<5% seeds surface colonized with scanty mycelial growth and no sporulation; 2= 5-25% seeds surface colonized with good mycelial growth and scanty sporulation; 3= 26-50% seeds surface colonized with good mycelial growth and good sporulation ; 4= > 50% seeds surface colonized with heavy sporulation [23]. *A. flavus* colonization Incidence determination

$$S= \frac{\textit{Seeds colonized}}{\textit{Total seed}} \times \mathbf{100}$$

2.6. Seed Coat Total Polyphenol Extraction and Quantification

Extraction: undamaged and matured seeds of all the studied genotypes were collected after drying. The seeds coats were removed from embryo before grinding to reduce in powder. After grinding, 0,1g of seed coat powder of each genotypes was introduced in the tube and added 10 ml of methanol (50%) before warming at 77°C for 1 hour. *Spectrometric Assay*: The total polyphenol was

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determined using FCR (Folin Ciocalteu reagent) [25].The absorbance was estimated at 750 nm using a Spectrophotometer (6715 UV/Vis JENWAY). The calibration curve was plotted using 0,1,2,3,4,5,6 ml of solutions of 0,05mg/ml of tannic acid concentration. The absorbance was measured to determine the content of total polyphenols using the following formula: C= (C1×V)/W with C being the content of total polyphenols expressed in mg equivalent tannic acid/g of dry matter, C1 the concentration of tannic acid established from the calibration curve in mg/L, V the volume of extract in L and W the weight of the plant extracting.

2.7. Genotypes Aflatoxin Content Quantification

Aflatoxin concentration in the seeds of fifty-five genotypes was estimated by an Enzyme Linked Immunosorbent Assay (ELIZA). In each trial (year1 and year2), 100 g of seeds were collected in the two water regimes plot. The 20g of this fine powder was used for the extraction of aflatoxin by dissolving in 70% (v/v) methanol containing 0.5% (w/v) KCl and homogenized and filtered through Whatman No1 filter paper. The filtrate was diluted 1:15 with methanol and used in duplicate to estimate aflatoxin concentration by indirect competitive ELIZA essentially as described by [26].

2.8. Analysis

The data used were the means of the two years experiments. GENSTAT 14th edition (VSN international Ltd. Hemel Hempstead, UK) was used to perform statical analysis. Shapiro-Wilk normality test was done before analysis of variance (ANOVA) which realised to assess the effects of genotype (G), water regime (W), and their interactions for the different traits measured. Microsoft Office Excel 2013 Software (Microsoft Corp., Redmond, WA, USA) was used to establish a relationship between the different yields, Aflatoxin content and the seed coat total polyphenol content. In addition it used to perfom the tables and figures.

Results

3.1. Yields and Yield Components

ANOVA revealed a significant genotype by water treatment interaction G× (Wtrt) for all yields traits (Table1) indicating that these parameters varied depending on the water regime. Water deficit imposed was more severe on seed number per plant and seed yield than on pod number per plant, pod yield and haulm yield (table2). Under well-watered conditions, the highest Pods Number per plant (PNP), and Seeds Number per plant (SNP) were observed on ICG 10950, ICG 11322, ICG 4598, ICG 1415, ICG 1519, ICG 332, ICG 5609, ICG 6407, ICG 3992 and ICG 12235. For pod yield (PY), seed yield (SY) and haulm yield (HY) under WW, the genotypes ICG 12697 (560.6 gm^{-2} , 376.8 gm^{-2} and 1022.4 g.m^{-2} respectively), ICG 12879 (472.2 gm^{-2}, 306.9 gm^{-2} and 584.7 gm^{-2} respectively), ICG 2019 (510.7 gm^{-2}, 289.6 gm^{-2} and 615.1 gm^{-2} respectively), ICG 4729 (393.3 gm^{-2}, 229 gm^{-2} and 711.7 gm^{-2}respectively), ICG 4543 (485.5 gm^{-2}, 227.7 gm^{-2} and 785.4 gm^{-2} respectively), ICG 4750 (580.1 gm^{-2}, 338 gm^{-2} and 764.5 gm^{-2} respectively), ICG 5195 (547.1 gm^{-2} , 302.3 gm^{-2} and 850.6 gm^{-2} respectively), ICG 5609 (524.9 gm^{-2}, 278.9 gm^{-2} , and 750.7 gm^{-2} respectively), ICG 6407 (522.4 gm^{-2} , 290.7 gm^{-2} and 833.9 gm^{-2} respectively) and ICG 6703 (546.3 g.m^{-2}, 306 g.m^{-2} and 932.7 g.m^{-2} respectively) were the highest performing (Table3).

However, ICG 10950, ICG 12235, ICG 1415, ICG 4598, ICG 5609, and ICG 6407 showed high performance under WW for pods number per plant (PNP), seeds number per plant (SNP) and low immature pods number per plant (IMPN). The latest genotypes revealed also the best in pods and seeds production with less immature pods under WS (Tablee3). In addition, the genotypes ICG 12879, ICG 14523, ICG 2019 and ICG 5195 showed high pods and seeds numbers per plant under WS. Our results showed that among the ten best performing genotypes under WS for pod yield, seed yield and haulm yield, five genotypes were also performing under Well-watered regime. These genotypes were ICG 12697 (363.4 gm^{-2}, 245.3 gm^{-2}, 513.6 gm^{-2}), ICG 12879 (351.7 gm^{-2}g.m^{-1}, 211.7 gm^{-2}, 536.9 gm^{-2}), ICG 2019 (384.1 gm^{-2} , 197.1 gm^{-2} , 474.3 gm^{-2}), ICG 4729 (329.7 gm^{-2} , 155.4 gm^{-2} , 530.2 gm^{-2}), and ICG 6703 (377.6 gm^{-2} , 213 gm^{-2} g.m^{-1}, 517.2 gm^{-2}). In addition, these five drought tolerant genotypes

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were followed by ICG 4598 (497.8 gm^{-2}, 197 gm^{-2}, 803 gm^{-2}), ICG 4684 (445.7 gm^{-2}, 246.8 gm^{-2}, 652.1 gm^{-2}), ICG 2106 (463.9 gm^{-2}, 239.7 gm^{-2}, 586.8 gm^{-2}), ICG 12988 (388 gm^{-2}, 206.5 gm^{-2}, 533.6 gm^{-2}), and ICGIL 11114 (442.2 gm^{-2}, 264.8 gm^{-2}, 627.4 gm^{-2}). However water deficit affected severely all yield components (PNP, SNP). Therefore, the pod and seed yields of the following genotypes are more severely affected by the intermittent water stress during seed filling phase by showing the highest seed yield loss (Table3). They are ICG 12235 (677 gm^{-2}, 183.4 gm^{-2}, 756.6 gm^{-2}), ICG 1142 (1247.9 gm^{-2}, 328 gm^{-2}, 666.6 gm^{-2}), ICG 14523 (746.5 gm^{-2}, 298.8 gm^{-2}, 780.2 gm^{-2}), ICG 14106 (776.9 gm^{-2}, 252.1 $g.m^{-2}$, 513.4 gm^{-2}), ICG 14630 (654.8 gm^{-2}, 150.6 gm^{-2}, 636.6 gm^{-2}), ICGIL 11125 (674.3 gm^{-2}, 207.8 $g.m^{-2}$, 594.2 gm^{-2}), and ICGIL17108 (582.3 gm^{-2}, 104.4 $g.m^{-2}$,808 gm^{-2}).

Table 1. Results of variance analysis (F value), F (probability), Means, LSD (least significant differences of means 5% level) of yield and its components. Genotype (G), Water Treatment (Wtrt) and genotype-by-water treatment interaction (G×Wtrt) effects were tested. Wtrt= water treatment; PNP=Pods Number per $plant^{-1}$; SNP= Seeds Number per $plant^{-1}$; IMPN= Immature Pods Number per $plant^{-1}$; HY=haulm yield; PY=pods yield; SY=Seeds Yield.

Sources	PNP		IMPN		SNP		HY (gm^{-2})		PY (gm^{-2})		SY (gm^{-2})	
of variance	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
F value	31.62	31.75	12	3.25	14.23	12	8.25	4.24	14	12	7.24	9.16
F (prob)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
WTrt	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
G×(Wtrt)	<.001		<.001		<.001		0.015		0.027		0.020	

Significance at 0.05, 0.01 and 0.001 level.

Table 2. Means variation of Yields and it's components under well-watered and water stressed treatments and the percentages of decreasing and increasing.

Traits	Water Traitements		WS negative effect (%)
	WW	WS	
Pods number per plant	**102±31**	80±25	21.92
Immature pods per plant (increasing)	9±5	21±10	57.14
Seeds number per plant	123±31	89±27	27.36
Pod Yield (gm^{-2})	678.54±203.51	546.15±163.5	19.43
Seed Yield (gm^{-2})	316.67±90.26	230.37±69.3	27.24
Haulm Yield (gm^{-2})	828.53±85.30	645.57±150.13	22.07

Table 3. Means variation of Yields and it's components under well-watered and water stressed treatments and Least significant differences 5% level (LSD). PNP: Pods number per plant; SNP: Seeds Number per plant; IMPN: Immature Pods Number per plant; HY: Haulm Yield; PY: Pod Yield, and SY: Seed Yield.

	Well-Watered						Water Stressed					
Genotypes	PNP	SNP	IMPN	PY (gm^{-2})	SY (gm^{-2})	HY (gm^{-2})	PNP	SNP	IMPN	PY (gm^{-2})	SY (gm^{-2})	HY (gm^{-2})
55-437	103±2	124±17	8±2	594.7±96	290.6±49	736.4±292	95±11	109±14	15±1	517±51	248.2±46	612.8±220

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	Well-Watered						Water Stressed					
Genotypes	PNP	SNP	IMPN	PY (gm^{-2})	SY (gm^{-2})	HY (gm^{-2})	PNP	SNP	IMPN	PY (gm^{-2})	SY (gm^{-2})	HY (gm^{-2})
Fleur 11	99±12	136±13	9±1	613.5±81	338.6±79	870.1±75	89±5	119±16	17±2	572±138	316.2±33	658±160
ICG 10950	98±5	112±19	4±1	701.6±61	376.8±70	1022.4±154	79±8	70±14	16±2	638.8±30	284.3±10	740.3±97
ICG 11322	76±22	72±12	4±1	567.8±33	272.8±39	691.6±118	62±14	65±9	26±2	529±49	242.3±31	583.9±127
ICG 1142	112±6	112±8	12±2	1664.9±255	369.4±78	798.6±69	84±10	78±4	23±2	1247.9±283	328±82	666.6±151
ICG 12235	207±16	116±18	34±3	766.6±154	188.5±32	930.1±82	141±6	56±13	76±3	677±128	183.4±17	756.6±148
ICG 12697	112±7	137±5	9±2	560.6±76	395.4±70	637.2±245	75±6	100±9	17±3	363.4±50	245.3±19	513.6±85
ICG 12879	101±2	129±11	8±2	472.2±68	306.9±27	584.7±184	86±6	64±20	16±2	351.7±33	211.7±48	536.9±58
ICG 12988	101±17	131±21	10±2	649.7±148	280.9±92	562.2±80	76±13	102±11	17±2	388±38	206.5±35	533.6±86
ICG 12991	115±8	153±21	4±1	525.3±42	243.8±36	657.5±203	79±10	86±4	17±3	443.9±33	199.5±25	629.5±169
ICG 13099	111±3	133±9	8±1	889.1±235	370.8±120	925.1±86	88±10	85±20	21±2	653.8±98	286.3±51	741.1±133
ICG 13603	177±12	205±26	10±1	780.9±83	356.2±111	775.4±58	134±9	163±20	25±1	662.5±131	256.6±17	589.9±102
ICG 13858	76±8	112±14	8±3	475.7±80	196.0±57	910.5±48	48±4	67±14	21±4	397.5±20	134.8±32	552.6±159
ICG 14106	92±8	142±17	8±2	833.5±47	321.4±35	724±162	53±8	74±13	19±2	776.9±145	252.1±50	513.4±50
ICG 1415	100±5	98±11	13±2	655.3±78	282.7±28	767.3±76	86±10	71±12	22±5	624.3±67	209.6±34	589.7±135
ICG 14523	159±13	174±12	10±2	906.0±154	378.6±31	954.3±58	121±12	111±3	21±3	746.5±27	298.8±25	780.2±125
ICG 14630	53±7	84±10	9±2	725.4±107	206.9±61	893.5±224	50±4	67±8	15±3	654.8±68	150.6±12	636.2±262
ICG 1519	86±5	79±15	8±2	590.5±96	214±55	761.5±185	63±11	70±16	20±3	485.6±16	180.9±36	695.4±208
ICG 156	102±14	125±11	9±1	682±84	237.9±24	1602±468	84±2	83±9	21±3	432.6±58	193.2±38	1074.3±406
ICG 163	104±7	113±10	10±3	733.9±204	433±83	1056.4±194	91±2	83±9	22±3	561.6±73	338±93	769.4±233
ICG 2019	69±7	105±16	11±3	510.7±145	289.6±82	615.1±108	37±25	49±24	20±4	384.1±85	197.1±73	474.3±274
ICG 2106	108±4	136±11	10±3	612.9±132	329.7±20	853.5±83	75±10	88±18	18±3	463.9±40	239.7±31	586.8±318
ICG 3027	98±11	130±9	11±3	752.9±50	381.7±41	892.4±104	62±2	82±19	15±2	613.9±81	304.3±49	718.2±151
ICG 311	106±9	135±10	7±2	638.5±106	393.6±71	676.2±124	86±8	101±11	14±1	562.8±102	269±20	543.1±54
ICG 332	63±1	83±8	5±2	658.8±26	283.4±34	985±60	39±5	47±11	18±2	457.5±44	162.1±37	726.5±144
ICG 334	105±9	159±20	8±1	884.7±22	444.3±29	813.5±172	98±7	143±15	24±2	692.3±89	286.5±32	553.5±138
ICG 36	100±13	112±15	7±2	778.8±64	222.2±35	737.3±111	53±7	67±11	27±3	528.2±83	148.6±13	463.2±110
ICG 3992	73±11	77±20	10±2	654.1±130	317.7±86	958.8±107	47±4	55±9	23±2	590.9±73	224.1±8	831.2±86
ICG 4543	89±11	126±14	4±5	485.4±56	227.7±19	785.4±122	73±6	87±18	18±1	385.3±67	183.4±52	578±141
ICG 4598	98±10	101±16	7±1	701.8±143	356.4±86	1050.8±62	76±7	89±18	19±1	497.8±41	197±9	803±66
ICG 4684	87±8	121±25	10±1	633.8±95	347.9±41	765.3±97	71±5	98±18	19±2	445.7±53	246.8±49	652.1±87
ICG 4729	80±2	94±6	10±2	393.3±64	229±17	711.7±115	66±7	80±13	23±1	329.7±47	155.4±28	530.2±91
ICG 4750	89±4	116±5	9±1	580.1±27	338±28	764.5±86	75±9	85±10	27±4	439.8±46	186.7±32	635±109
ICG 4764	84±7	125±7	8±2	727.3±157	369.3±68	839.8±200	79±3	108±7	25±3	549.6±104	231.5±24	643.7±165
ICG 513	100±14	138±24	12±4	861.2±101	471.9±98	815.3±98	85±6	110±22	29±2	688.8±65	306±12	696.7±150

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	Well-Watered						Water Stressed					
Genotypes	**PNP**	**SNP**	**IMPN**	**PY (gm^{-2})**	**SY (gm^{-2})**	**HY (gm^{-2})**	**PNP**	**SNP**	**IMPN**	**PY (gm^{-2})**	**SY (gm^{-2})**	**HY (gm^{-2})**
ICG 5195	134±11	174±12	11±2	547.1±21	302.3±29	850.6±27	98±8	100±6	25±2	536±51	264.4±82	708.2±185
ICG 532	90±3	114±7	10±1	729.9±123	298.2±55	824.7±145	71±8	104±2	18±2	532.4±96	213.1±60	677.5±110
ICG 5609	60±6	98±21	6±2	524.9±81	278.9±14	750.7±171	49±10	58±9	15±3	411.5±34	146.8±17	556.8±147
ICG 5663	144±9	135±6	17±2	667.1±78	313.9±64	735.7±2116	91±13	85±8	26±3	541.8±59	212.8±51	569.4±64
ICG 6263	76±11	89±14	8±2	550.2±41	152.1±43	646.5±131	53±7	64±15	21±3	486.8±44	86.1±11	402±117
ICG 6407	62±4	79±7	7±3	522.4±52	290.7±17	833.9±96	52±3	67±20	19±2	500.9±45	236.1±34	741±110
ICG 6654	89±13	138±8	9±2	687.6±94	342.7±71	697.3±121	70±6	99±6	18±2	630.1±77	265.3±33	596±125
ICG 6703	92±7	116±16	7±2	546.3±50	306±44	932.7±104	79±5	100±16	12±2	377.6±40	213±23	517.2±111
ICG 6813	138±9	172±14	11±2	732.6±68	377.9±18	744.1±429	125±14	116±3	25±2	634.7±56	343.3±9	751.8±98
ICG 6888	66±9	80±2	6±2	540.8±64	259.9±81	830.6±193	53±5	67±5	36±2	417.5±26	154.4±22	657±148
ICG 7181	69±5	91±15	10±2	567.4±51	310.9±51	908.5±89	62±10	65±5	22±3	470.5±77	220.3±40	757±105
ICG 721	106±8	132±21	11±2	804.5±75	409.4±33	1064.5±83	96±10	123±19	15±2	638.1±44	321.2±46	954.4±86
ICG 76	149±22	162±10	9±2	933.6±51	467.7±69	807.4±106	132±9	131±18	19±3	727.2±72	342.7±41	618±155
ICGIL 11102	141±31	159±29	8±2	603.7±62	282.9±62	974.6±139	119±8	127±21	23±2	568.6±56	248.3±62	761.1±177
ICGIL 11110	132±13	151±8	12±2	833±102	418.1±55	848.4±120	89±9	103±18	25±2	578.8±132	270.8±29	567.8±224
ICGIL 11114	70±9	109±22	5±1	712±110	423.5±85	825.2±158	65±5	91±19	21±3	442.2±135	264.8±32	627.4±182
ICGIL 11125	121±4	132±12	11±1	724.4±119	398.2±56	728.5±116	105±10	108±5	19±2	674.3±194	207.8±61	594.2±184
ICGIL 17108	135±7	131±5	15±1	765.7±98	180.7±35	1022.2±111	115±6	89±9	21±2	582.3±52	104.3±23	808±181
J 11	116±8	127±9	8±1	497.4±39	259.1±39	697.3±61	79±8	87±8	17±2	443.7±70	205.3±13	493.5±183
JL24	105±9	140±19	13±2	565.8±69	280.1±37	720.6±33	88±3	126±26	28±2	488.5±30	245±52	539.2±214
Means	**102±31**	**123±31**	**9±4**	**678.5±204**	**316.7±90**	**828.5±279**	**80±25**	**89±27**	**22±8**	**546.2±164**	**230.4±69**	**645.6±215**
LSD	**15**	**21**	**5**	**131**	**79**	**145**	**12**	**20**	**9**	**107**	**56**	**140**

3.2. Intermittent Water Deficit (WS) Effect on Pre-Harvest Aflatoxin Contamination (AC)

Analysis of variance revealed a significant genotype by water treatment interaction (G×Wtrt) for aflatoxin contamiation under well-watered and water stressed treatments (Table4). Under WW conditions, the lowest aflatoxin contamination were observed on five genotypes, ICG 12988 (0.28 ppb), ICGIL 17108 (0.30 ppb), ICG 5195 (0.40 ppb), ICGIL 11110 (0.46 ppb) and ICG 311(0.48). While ICG 332 (6.80 ppb), ICG 11542 (5.56 ppb), Fleur11 (4.01 ppb) and ICGIL11114 (3.75 ppb) showed the highest aflatoxin contamination under WW treatment (Figure1). Intermittent water stress (WS) imposed during seed filling phase increased aflatoxin contamination up to 67.16% (Figure2). Under water deficit the highest aflatoxin content were observed on ICG 332 (19.44 ppb), ICG 6703 (18.65 ppb), ICG 4764 (13.03 ppb), JL24 (11.37 ppb) and ICG 11542 (10.88 ppb) (Figure2). These genotypes were revealed more sensitive to aflatoxin contamination under drought. While, ICG 311 (0.83 ppb, ICG 2119 (0.90 ppb), ICGIL 17108 (1.6 ppb), ICG 4684 (1.93 ppb) and 55-437 (2.15 ppb) showed the

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lowest aflatoxin content under drought conditions. These genotypes were resistant to aflatoxin contamination (AC) under WS (Figure2).

Table 4. Results of variance analysis (F value), F (probability), Means, SLD (least significant differences of means 5% level) of aflatoxin contamination under Well-watered and Stressed treatments.

	Aflatoxin B1	
Genotypes	**WW**	**WS**
Means	1.81	5.51
LSD	1.14	1.49
F value	9.55	56
F (Prob)	<.001	<.001
G×W(Trt)	<.001	

Significance at 0.05, 0.01 and 0.001 level.

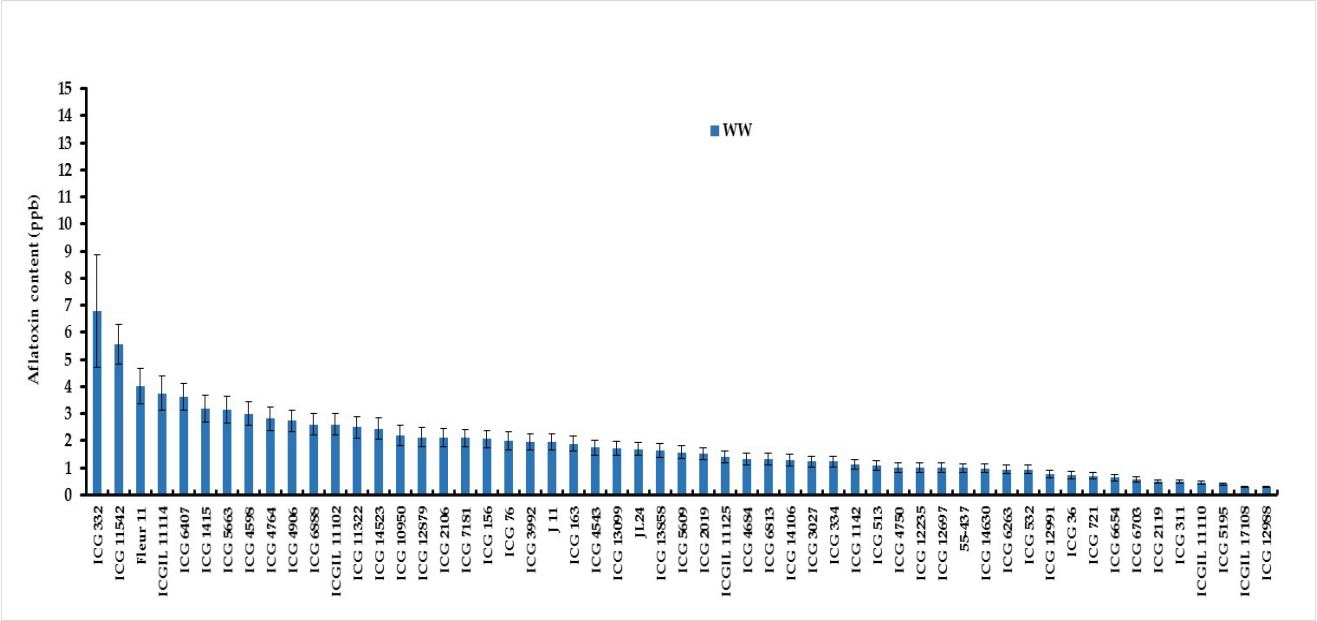

Figure 1. Aflatoxin content (ppb) variation of fifty-five peanut genotypes under well-watered (WW) treatment.

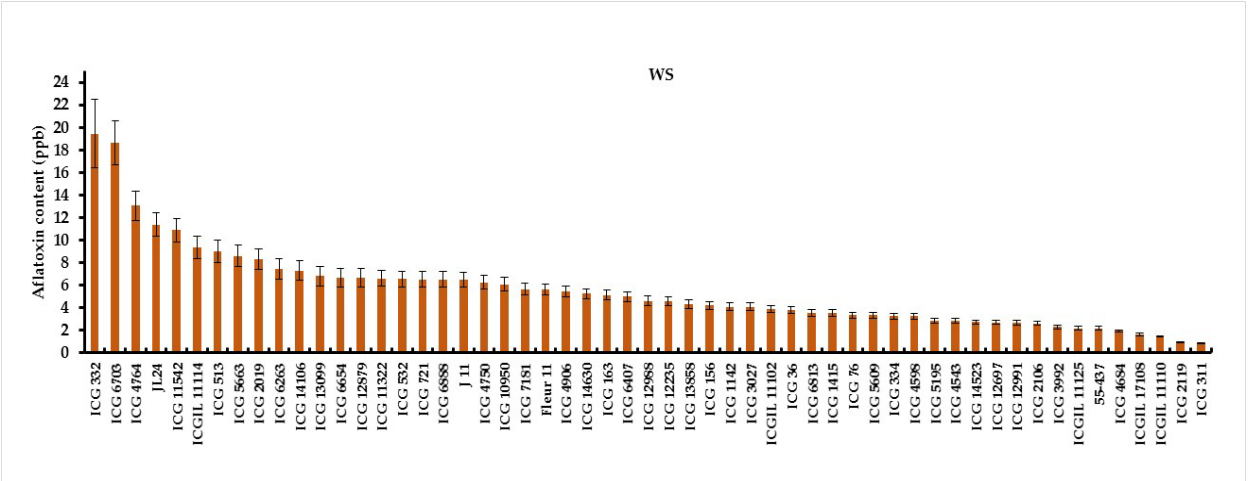

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Figure 2. Aflatoxin content (ppb) variation of fifty-five peanut genotypes under water stressed (WS) treatment.

3.3. Seeds Colonisation and Total Polyphenol Content of Seed Coat

ANOVA revealed a significant (P<0.001) genotypic difference for *A. flavus* infestation rating. The total polyphenol content of seed coat showed also a significant genotypic variation (P<0.001) (Table5). Tow genotypes ICG 12988 (18%), ICG 311(19%) showed *A. flavus* infestation as well as the resistant check 55-437(21%). These genotypes with 55-437 were the less infested (rate <25%). This indicated their resistance to *A. flavus* colonisation. They were followed by ICG 4543 (28.33%), ICG 11542(28.33%), J11 (40%) and ICG 76(42.5%) that showed *A. flavus* infestation rate less than 50%. The remaining genotypes showed *A. flavus* infestation rating more than 50% indicating high susceptibility to *A. flavus* colonization (Table6). The highest total polyphenol content of seed coat was observed on ICGIL 17108 (62.96%), ICG 3027 (57.50%), ICG 14630 (56.95%), ICG 12235 (56.67%) and ICGIL11125 (56%30%). All the tested genotypes showed more than 50% *A. flavus* infestation rating. While, the lowest seed coat %TPP content was showed by ICG 532 (42.74%), ICG 1415(43.68%), ICG 5609 (43.74%), and ICG 11542 (43.90%) (Table6). Then only ICG 11542 showed less than 50% of *A. flavus* colonization rating.

Table 5. : Results of variance analysis (F value), F (probability), Means, LSD (least significant differences of means 5% level) of the Percentage of seed coat Total Polyphenol (%TPP) and the percentage of *Aspergillus Flavus* Colonisation.

Genotypes	%TPP	% AFC
Means	50.50	85
LSD	2.54	16.63
F value	19.38	4.12
F (Prob)	<.001	<.001

Significance at 0.05, 0.01 and 0.001 level.

Table 6. Categorisation of fifty-five genotypes according to the percentage of *A. flavus* colonization incidence and the percentage of seed coat total polyphenol content. CI= Colonization incidence; Percentage of *A. flavus* colonization = % AFC; percentage of Total Polyphenol =%TPP.

	Genotypes	% AFC	%TPP	Genotypes	% AFC	%TPP	Genotypes	% AFC	%TPP
CI ≤25%	ICG 311	19	53.08	55-437	21	49	ICG 12988	18	52.1
CI>25%≤50%	ICG 11322	50	50.43	ICG 14523	43	51.61	ICG 14523	43	51.61
	ICG 11542	28	43.90	ICG 163	45	47.38	ICG 3027	83	57.50
	ICG 4543	28	47.55	ICG 76	42	52.57	J 11	40	55.16
CI >50%	Fleur 11	86	47.90	ICG 10950	91	56.05	ICG 1142	100	50.32
	ICG 12235	85	56.67	ICG 12697	91	51.47	ICG 12879	100	45.50
	ICG 12991	81	48.75	ICG 13099	66	50.35	ICG 4906	83	46.86
	ICG 13858	91	53.27	ICG 14106	100	48.76	ICG 1415	100	43.68
	ICG 14630	81	56.95	ICG 14630	81	56.95	ICG 156	90	51.86
	ICG 2019	100	52.34	ICG 2106	83	48.63	ICG 332	100	53.22
	ICG 334	66	50.76	ICG 36	66	49.14	ICG 3992	83	53.17
	ICG 4598	100	48.16	ICG 4684	75	45.97	ICG 2119	100	52.41
	ICG 4750	65	47.38	ICG 4764	70	50.23	ICG 513	100	53.69
	ICG 5195	100	52.36	ICG 532	100	42.74	ICG 5609	83	43.74
	ICG 5663	81	49.36	ICG 6263	91	54.01	ICG 6407	61	52.14
	ICG 6654	91	48.31	ICG 6703	91	46.32	ICG 6813	46	46.53
	ICG 6888	83	46.09	ICG 7181	100	45.92	ICG 721	91	50.20

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ICGIL 11102	100	53.09	ICGIL 11110	75	51.29	ICGIL 11114	75	54.34
ICGIL 11125	61	56.30	ICGIL 17108	91	62.96	JL24	100	47.93

3.4. Relationship between Aflatoxin Contaminations with Total Polyphenol, Pod Yield, Seed Yield, and Haulm Yield

Aflatoxin contamination in resistant genotypes such as ICG 1415, ICG 2106, ICG 311, ICG 4684, ICG 4543 and the resistant check 55-437 showed a strong positive correlation with the seed coat total polyphenol content (r^2=0.80; 0.82), the pod yield (r^2=0.58; 0.14), seed yield (r^2=0.74; 0.31) and haulm yield (r^2= 0.47; 0.39) under WW and WS treatments respectively (Figure 3 and Figure 4).

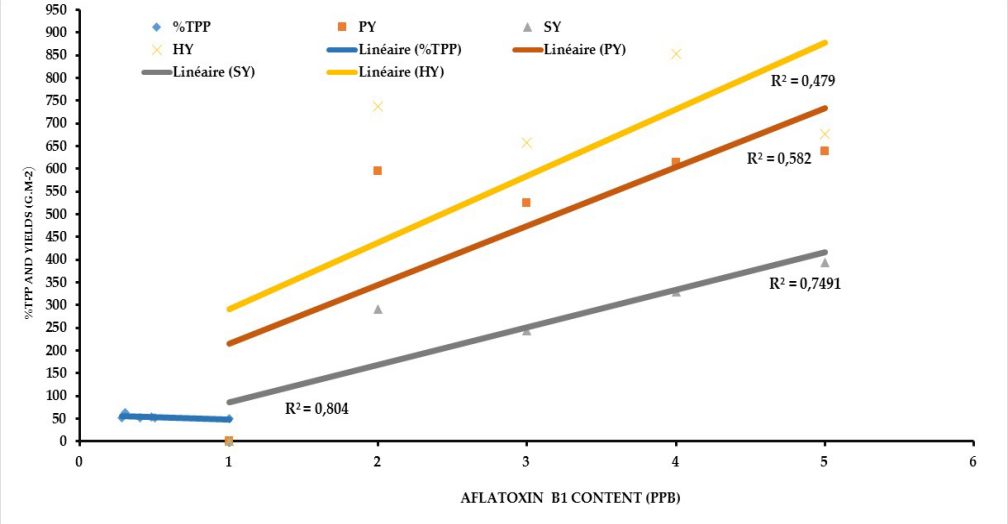

Figure 3. Relationship between Aflatoxin contamination and Seed Coat total polyphenol content, Pod Yield, Seed Yield, Haulm Yield under Well-Watered conditions (WW).

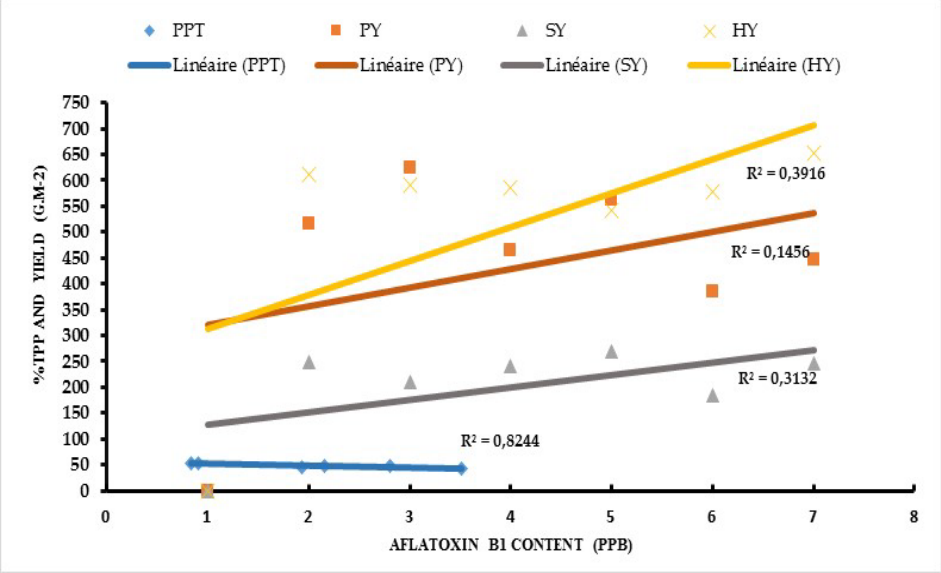

Figure4: Relationship between Aflatoxin contamination and Seed Coat total polyphenol content, Pod Yield, Seed Yield, Haulm Yield under Stressed conditions (WS).

4. Discussion

This study revealed a wide genotypic variation for all studied traits under two water treatments. The effect of genotype and water treatment interaction (G×Wtrt) showed that genotype's performance differed under well-watered treatment (WW) and water stressed (WS) treatments.

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Indeed, pod yield (PY), seed yield (SY), and haulm yield (HY) were reduced by intermittent water deficit during seed filling up to 19.49%, 27.24% and 22.07% respectively. As for the immature pods number per plant (IMPN) and aflatoxin contamination (AC), they have increased up to 57.14% and 67.16% under WS. This indicated that intermittent drought applied from beginning seed filling to maturity reduced yields, it's components while increased aflatoxin contamination and immature pods number. This results are similar to the previous studies which reported that water deficit imposed during 60 to 85 DAS lead to 26 % yield loss [27]. It also corroborates with others works that reported 24% of yield reduction during the end of growing season [9,28]. The top ten drought tolerant genotypes revealed in this study are: ICG 12697, ICG 12879, ICG 2019, ICG 2119, ICG 6703, ICG 4598, ICG 4684, ICG 2106, ICG 12988, and ICGIL 11114. These genotypes showed high yields under WS. This tolerance can be explained by their ability to partition dry matter into harvestable yields under limited water supply [29]. Interestingly, five (5) genotypes among these top ten drought tolerants showed low aflatoxin contamination under drought. They are: ICG 12697(2.68 ppb), ICG 2119 (0.90 ppb), ICG 4598 (3.23 ppb), ICG 4684(1.93 ppb), and ICG 2106(2.60 ppb). This indicated that these five genotypes maintained high yields under water deficit and kept intact their seed coat which protected seeds to *A. flavus* invasion and aflatoxin contamination. Previous studies reported that high aflatoxin levels are usually found in damaged pods compared to pods with intact shells [30]. Thus our findings are similar to the previous results which indicated that high pod yield under drought conditions is related to low seed infection and low aflatoxin contamination [31–33].

These last five drought tolerant and aflatoxin resistant genotypes are belonging to Spanish and Valencia botanical types. This suggested that peanut Spanish and Valencia botanical types are more resistant to aflatoxin contamination under intermittent drought during seed filling phase than Virginia type. Because of Virginia type has a long seed filling phase which exposed it more to the risk of water stress that promote pod damage, seed coat crack, *A. flavus* invasion and aflatoxin contamination while Spanish and Valencia types have a short seed filling phase. In addition to the top ten drought tolerant and low AC under WS, the genotypes ICG 311 (0.3ppb), ICG 14523 (2.71ppb), ICG 4543 (2.80ppb), ICG 5195 (2.83ppb), ICG 6813 (3.51ppb), and ICG 1415 (3.51ppb) showed also low aflatoxin content as the resistant check 55-437 (2.15ppb). Indeed, water deficit allowed identifying the genotypes having a specific tolerance to water deficit and keeping intact their seed coat that limited *A. flavus* invasion and aflatoxin contamination.

This suggest that water deficit injuries to the pods and testa enables the fungus to enter and infect the kernels [7,30,34]. Recent studies had the same conclusion for the low aflatoxin level of these genotypes under field conditions [21] and under different level of drought and temperature [35]. Furthermore, this study reveal the genotypes which content low aflatoxin content under the limitation of the European Union commission standard of total aflatoxin (4ppb) for human consumption [36]. Then, others authors reported drought tolerant genotypes have been reported to have tolerance to aflatoxin contamination [9,37].

In this study, the singnificant difference of the seed coat total polyphenol (%TPP) and the incidence of *A. flavus* colonization among genotypes could be due to the diversity of seed coat color, physical and chemical components. These results revealed that seed coat total polyphenol content reduced more *A. flavus* colonization acording to the seed coat color than to the quantity of total polyphenol in the seed coat. However, the variations of the incidence of *A. flavus* colonization and the percentage of total polyphenol (%TPP) were observed mainly on the genotypes ICG 311 (19%; 53.07%), ICG 2119 (100%; 52.14%), ICG 4684 (75%; 45.97%), ICG 14523 (43%; 51.61%), ICG 4543 (28%; 47.55%), ICG 5195 (100%; 52.36%), ICG 6813 (46%; 46.53%), and ICG 1415 (100%; 43.68%) respectively. These results suggests that the percentage of total polyphenol did consistently affect aflatoxin contamination. It is in agreement with several studies which reported that other phenolic content and cell wall must have effect with total polyphenol to inhibit pre-harvest aflatoxin contamination [18,19]. Thus, the pink small grain such as 55-437 that showed low incidence of *A. Flavus* colonization (21%) and low percentage of seed coat total polyphenol (49%) resisted more to *A. flavus* colonization than the dark big grain such us ICG 12235 that showed 85% of incidence of *A.Flavus* colonisation and 56.67% of seed coat total polyphenol. Furthermore, aflatoxin contamination under intermittent

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drought was significantly associated with the seed coat total polyphenol content and seed size. These results corroborate with the previous studies which reported that the difference in mycelial growth surface coverage can be explained by the difference in physical and chemical features of the seed-coat, pod-shell thickness and reticulation [19,38–40]. The peanut seed coat is composed of multiple cell wall layers, and peanut varieties differ in composition of flavonoids and tannins which ultimately give different colour to the peanut seed coat [18,19,41].

In this study, the following drought tolerant genotypes ICG 1415, ICG 2106, ICG 311, ICG 4684, ICG 4543 revealed significant relationship between aflatoxin resistance and seed coat total polyphenol under two water treatments. (r^2=0.80; r^2=0.82). It's also associated with pod yield (r^2=0.58; r^2=0.14), seed yield (r^2=0.74; r^2=0.31), and haulm yield (r^2= 0.47; r^2=0.39) respectively under WW and WS. This indicated that these drought tolerant genotypes kept their seed coat intact and minimized aflatoxin contamination under intermittent drought. Thereby our findings are similar to the previous studies that reported the existence of seed coat resistance was a logical assumption, considering that seeds with damage testa are more easily and rapidly invaded by fungus than those with intact testa, and coloured testa conferred greater resistance to invasion by *A. flavus* than white or variegated testa [42,43].

5. Conclusions

Our findings showed that intermittent water deficit during seed filling phase decrease significantly peanut yields and increase aflatoxin contamination. The peanut genotypes with pink color of seed coat belonging to Spanish and Valencia botanical type are more resistants against *A. flavus* invasion and aflatoxin contamination than Virginia botanical type under intermittent water deficit because of their short seed filling phase. From this study, the genotypes ICG 12697, ICG 2119, ICG 4684, ICG 2106, ICG 311, ICG 4543, ICG 5195 and ICG 1415 showed the best performance for drought tolerance and aflatoxin minimizing. These genotypes can be used in breeding program to select the varieties that combine drought tolerance and minimize aflatoxin contamination in the semi-arid tropic.

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