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Research Article

Physiological Response and Aflatoxin Contamination in Maize (*Zea Mays* L.) Grown Under Varying Water-Deficit Levels in a Semi-Arid Region

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ABSTRACT

The present study explored the effects of water deficit levels at different growth stages of maize (*Zea mays* L.) cultivated in semi-arid conditions with regard to physiological responses and aflatoxin contamination. Thus, in addition to physiological responses, the objective of the study is to investigate the effect of water deficit conditions on aflatoxin contamination on the yield of four types of hybrid maize kernels during the kernel-filling period. The physiological growth changes of maize hybrids were gauged for parameters viz. relative water content (RWC, %), root-shoot ratio (R/S), and specific leaf area (SLA). At the same time, the study used HELICA total Aflatoxin Assay for estimating aflatoxin contamination in the produced maize kernel. Results revealed that the third level of water-deficit, which extended to the dent stage, led to lower RWC%, and higher (R/S), alongside lower (SLA). Although the aflatoxin level was estimated to be 18.5 ppb in the maize kernels (FDA limit = 20 ppb), it was apparent that cultivation in a semi-arid condition runs the risk of aflatoxin contamination, likely due to water shortage and high growing temperatures.

Keywords: Aspergillus flavus, Corn, Drought, Relative water content, Root-shoot ratio, Specific leaf area,

Introduction

The physiological responses of plants to a gradient of soil moisture content can help determine the soil moisture level at which water deficit stress in plants begins. Any maize variety's performance depends on its genetic make-up and response of the desirable characteristic under stress and non-stress situations, which may then be used to calculate how much water can be reduced without impairing the development and production of the crop [1]. Low soil moisture levels on growing crops typically manifest as decreased photosynthesis in affected plants. Other parameters observed include the concentration of photosynthetic and photosynthesis pigments, chlorophyll fluorescence, and relative water content[2]. Relative water content (RWC) is a key indicator of water adequacy in growing plants and the physiological processes in their cells. For instance, the RWC is higher at the initial stages of leaf development and gradually declines as dry matter accumulates and the leaf matures. Also, RWC is intimately related to water uptake by roots and water loss via transpiration. High temperature-related water deficit in plants reduces the corresponding leaves' water potential, RWC, and transpiration rate [3]. RWC is affected by the interplay between drought intensity and the species [4].

Pertinently, maize grown under water-deficit conditions shows significantly reduced leaf area, and the outcome is exacerbated with increased severity of water deficiency. Such conditions profoundly lower the relative water content, negatively affecting cell division and leaf elongation [5]. Another parameter to contemplate is the

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specific leaf area (SLA), which correlates with the ratio of leaf area expansion and dry matter of the leaves. Changes in water content in these cells can affect maize development, related to the leaf area and their net assimilation rate. Therefore, a large reduction in cell water potential directly affects the turgor pressure of the leaf cells, thus inhibiting leaf expansion [6]. Maize grown under water-deficit conditions reportedly have lower SLA values due to poor biomass accumulation [7]. Another physiological parameter used to gauge plant growth under water duress is the ratio between the root and shoot (root/shoot, R/S), whereby a ratio of R/S indicates a favorable growing condition and adaptation of crops to water-deficit situations. Also, differences in plant genotypes and environmental conditions yield varying R/S ratios, whereby lower ratios are observed in maize grown in water-deficit conditions [8]. This has to do with the dry soil conditions impacting the growth of their roots and shoots [9].

Research has shown that pests can damage maize kernels in the field and during storage. As a matter of fact, fungi are among the microbial pests of concern, as their infestation can render grains unsuitable for human or animal consumption. Certain fungi produce aflatoxins, which pose serious health risks to humans and animals with types B1, B2, B2a, G2, G2a, M1, M2, P1, Q1, and R0 being the most significant. Aflatoxins are highly carcinogenic mycotoxins produced by two parasitic fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, under water-deficit and high-temperature conditions. In fact, aflatoxin B1 is the most prevalent, incredibly carcinogenic to some animal species, and has been isolated in maize kernels. These fungi can infect growing maize during the filling period of different grain crops and counting [10]. The Food and Drug Administration specified that 20 ppb is the threshold of aflatoxin contamination for marketable maize grains. At the same time, those exceeding 300 ppb must be combined with corn having little or no aflatoxin before they are permitted for cattle consumption [11]. Economic losses from aflatoxin poisoning could amount to hundreds of millions in the agricultural sector. The Food and Agriculture Organization reported that aflatoxins had damaged 25% of the world's food crops, aggravated by high temperature, pH, drought stress, and other external environmental parameters that promote aflatoxin production and increase their toxicity. [12-13]

The physiological stages of the maize kernel play a major role in regulating mycotoxin production [14]. To date, aflatoxin contamination has been detected in 49% of maize samples, where 15% of the total samples exceeded the permitted level (20 ppb). Notably, 65% of maize samples from drought-prone regions exceeded the permissible limit [15]. Irrigation decreases aflatoxin contamination in growing maize, while drought-tolerant varieties have lower aflatoxin levels when grown under water-stress conditions [16]. There is substantial evidence that drought increases aflatoxin levels [17] because the pathogenic *A. flavus* and *A. parasiticus* grow best at 30°C. Likewise, sterigmatocystin production, a precursor to aflatoxin by *A. nidulans*, is optimal at 37°C [18-19]. There are strategies to limit aflatoxin and fumonisin contamination in harvested kernels and commercial maize hybrids, namely, by crop planting during a key kernel-filling stage, particularly during a non-drought or low-temperature season [20]. This strategy is usually effective during the critical kernel-filling duration phase, between silking and physiological maturity [21].

Hence, it is clear that microclimatic conditions can profoundly increase aflatoxin contamination frequency in maize [22]. However, kernel contamination incidences are lower during shorter, colder days in late September and October because of reduced photosynthesis and increased plant senescence [23]. Hence, the study's objective is to investigate the effect of water deficit conditions on the physiological performance of four types of hybrid maize and of aflatoxin contamination of kernel yield during the filling period in a semi-arid region.

Material and Methods

The growth physiology, yield, and the percentage of aflatoxin contamination in four maize hybrids were studied using two different field experiments under climatic conditions in Kalar district (Lat. 34° 38` 44`` N, long. 45° 19'21" E, 231m meters above sea level (m.a.s.l.). Experiments were carried out during the spring and autumn seasons of 2021. The region is semi-arid, characterized by mild winters (250 – 300 mm rainfall) and hot summer. Table 1 denotes meteorological data for both growing seasons from April to October 2021. The four maize hybrids were treated with three different levels of regulated water deficit and were adjusted as follows:

1) I_1 = Full irrigation based on crop water requirement across its life cycle.
2) I_2 = Skipping every other irrigation from (V5) pre-silking to Blister or milking stage, and no irrigation one-week post-silking (R3).
2. I_3 = No irrigation from (V5) pre-silking to Dent stage (R5); differences between I_2 and I_3 were estimated after two weeks. The water deficit levels in this study were organized to include the seed formation stages during the seed-filling phase, whereby the seed's components undergo physiological shifts due to changes in pH and the rate of amylopectin. The temperatures during the investigations were between 25.81 to 41.55°C, shown in Table 1.

To estimate the hybrid's susceptibility to infection, the degree of infection by the fungi was correlated to the irrigation levels.

Split-plot design was used in both field experiments with three replications. In this study, the irrigation levels were the main treatment, and the four maize hybrids were the sub-plot treatments. The plants were cultivated at 0.7 m between rows and 0.25 m between every two plants within rows. The seeding dates for the two different seasons were April 7 and July 5 for the spring and autumn of 2021, respectively. The physical and chemical properties of the experiment soil samples are shown in Table 2. In the study, 200 kg.ha^{-1} nitrogen fertilizer was applied as recommended. Elemental analysis of soil by ICP-OES in PPM as 46% urea, while T.S.P 48% was the basal dose for phosphorous. All required agricultural processes were applied in both seasons except the irrigation, as described above.

Meanwhile, the soil's field capacity and wilting point were estimated by a gravimetric method to correlate with the pre-determined irrigation schedule. The soil moisture content was measured by weighing a subsample of a fresh, sieved composite sample or fresh soil. The sample was then oven-dried until a consistent mass was achieved and then reweighed. The moisture content was expressed as the mass of water per mass of dry soil, using the following Equation 1:

Soil moisture (g water per g dry soil) =
(fresh weight – dry weight) / dry weight (1)

To demonstrate the illustrative responses of the four maize hybrids versus the water deficit levels, the relative water content of the leaf was measured at different growth stages from V5 to PM. The estimation of the root-shoot ratio and specific

Table 1. Meteorological data (max,min,av temp. and max, min, av relative humidity, and reference evapotranspiration) of Kalar district for both growing seasons in the spring and summer of 2021

Months	T_{max}	T_{min}	$T_{av.}$	RH_{max}	RH_{min}	RH_{av}	ETo
April	26.520	25.090	25.805	38.500	33.300	35.800	5.800
May	29.600	28.270	28.935	21.100	17.600	19.200	8.100
June	40.000	32.590	36.295	13.600	10.800	12.000	9.800
July	44.000	38.120	41.060	39.700	38.100	38.900	10.100
August	44.900	38.200	41.550	12.800	10.00	11.100	9.500
September	38.870	37.400	38.135	28.400	7.800	16.100	7.700
October	32.650	29.700	31.175	28.780	8.920	17.180	6.890

Note: AV: average, Max: maximum; Min: minimum

Table 2. Physical and chemical properties of agricultural soils of the experiment site

Soil Texture			Soil Properties						
Sandy silty loam									
Clay (%)	**Silt (%)**	**Sand (%)**	**PH**	**EC Ms/cm**	**Lime CaCO3 (%)**	**Organic Matter (%)**	**Nitrogen %**	**Available K_2O (ppm)**	**Available P_2O_5 (ppm)**
10.310	30.900	58.790	8.160	913.000	41.410	1.150	0.220	112.000	7.800

Soluble Cations and AnionsMeq./L.									
AL	B	NI	K	SI	Na	Ti	Cr	Sr	Ba
3.522	64.328	122.307	6,210	322.000	213.990	881.263	165.272	335.457	167.847

leaf area of maize hybrids, as the most relevant criteria to gauge the effect of water content on the growing maize, was estimated using the following Equations 2 to 4:

$$Relative\ Water\ Content\ (\%) = \frac{(FW-DW)}{(TW-DW)} x\ 100 \quad (2)$$

The specific leaf area (SLA) was estimated by using Equation 3 [24]

$$Specific\ leaf\ area = \frac{Leaf\ area/\ Plant\ (cm2)}{Leaf\ Weight/Plant\ (g)} \quad (3)$$

For calculating the root-shoot ratio, the following Equation 4 was used:

$$Root - Shoot\ ratio = \frac{Root\ Dry\ Weight\ (g)}{Shoot\ Dry\ weight\ (g)} \quad (4)$$

{Leaf area = Leaf length (cm) × Leaf width (cm) × 0.74} (5)

Also, Equation 5 was used to estimate the leaf area per plant. The accumulation rates of dry matter-to-shoot throughout the seasons were measured using different fresh samples (including shoot or plant parts above the soil surface). All samples were freshly collected and split into individual categories before weighing. Each sample was then oven-dried at 70°C until completely dry and weighed.

The HELICA Total Aflatoxin Assay was performed on randomized kernel samples taken from mature maize hybrids grown in spring and summer to estimate the percentage of aflatoxin contamination. This competitive enzyme-linked immune assay is intended to detect aflatoxin B1 quantitatively in B2, G1, and G2 in kernels [25]. Data from the two field experiments were statistically analyzed using Genstat - version 12.1 software. The significant differences in the treatment means were compared using Least Significant Difference (LSD) test at P< 0.05.

Results and Discussion

Relative water content

It is worth mentioning here that the RWC has a vital role as an indicator of the plant water status. Table 3 enlists the influence of water deficit levels on the RWC of maize plants in spring and summer. The samples were monitored at two weeks intervals after the vegetative growth stage (V5). As it can be seen, the RWC of maize plant leaves decreased with increased severity of water deficit levels from I_1 to I_2 and I_3, as well as during pre-silking, silking, and post-silking stages at 45, 60, and 75 days after sowing (DAS), respectively. Contrariwise, the lowest RWC was observed at I_3 in all developmental stages. Hence, the outcome of this study and the growth of the maize plants in both seasons are seen to agree with the result of a previous report by Cleveland et al. [18].

Despite the adaptability of these maize hybrids to different stress factors, their RWC percentages did vary at different growth stages during both seasons (Table 4). RWC values in the four maize

Table 3. Changes in RWC values at different growth stages with respect to varying in water deficit conditions in spring- and autumn-grown maize hybrids.

Irrigation program	RWC % in spring			RWC % in autumn		
	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS
I_1	70.300	74.900	72.200	72.600	71.900	59.200
I_2	69.400	74.500	69.500	70.100	70.380	56.900
I_3	64.800	71.200	68.100	67.200	68.810	56.200
L.S.D	6.690	9.610	5.660	2.880	5.110	2.880

Table 4. Relative water content (RWC%) at different growth stages in spring- and autumn growing seasons for all maize hybrids

Maize Hybrids	RWC % in spring			RWC % in autumn		
	45DAS	60DAS	75DAS	45DAS	60DAS	75DAS
H1	65.200	71.800	67.000	71.000	70.230	55.000
H2	69.500	74.300	70.500	69.100	70.300	61.300
H3	76.000	75.300	67.800	73.100	69.180	56.000
H4	69.200	72.600	67.300	66.700	71.740	57.400
L.S.D	13.630	4.390	4.750	12.890	5.691	10.120

hybrids concomitantly increased with increasing water shortages following the growth changes from the pre-silking stage (45 DAS) to the near-silking stage and leading up to the post-silking stage (75 DAS). Maize hybrids demonstrated the lowest RWC values, particularly at the reproductive growth stages. The outcome seen here was likely due to the onset of kernel setting, plus the substantial decline in soil moisture that diminished the photosynthesis process. However, the study results varied from one maize hybrid to another, probably due to their disparities in susceptibility toward growing in water-deficit environments. Hybrids H_2 and H_3 manifested higher RWC values than H_1 and H_4 in all growth stages when grown at similar water deficit levels. Aside from differences in RWC responses in growing maize hybrids concerning the crop's growth stage during water shortages, other contributing factors include the degree and duration of the maize hybrids exposed to water-deficit conditions [1].

Root-shoot ratio

The study found that the rate of accumulated dry matter that was partitioned to the roots or shot was responsible for the differences in the root-shoot ratio (R:S) in maize plants grown in both seasons. Figure 1 reveals the significant effect of water deficit levels I_2 and I_3 ($p < 0.05$) compared to a well-irrigated condition (I_1) in the spring. The latter exhibited the lowest R/S ratio (0.055) under I_2, while the highest was observed for maize grown under I_1. Smaller changes in root growth were noted for maize grown with increased water-deficit conditions compared to ones which grew under adequate water conditions.

In this study, the water deficit conditions represented by I_2 and I_3, plus the high daily temperature throughout the spring growing season, might have promoted the shift in the growth pattern from shoot growth to root growth. The water-deficit-affected maize responded by undergoing a net assimilation rate that favors the root region [1]. This is, in fact, the plant's adaptation to grow under water-deficit condition (water-deficit levels I_2 and I_3). The altered pattern of biomass accumulation in the maize samples was supported by the significant differences in their SLA results, as shown in

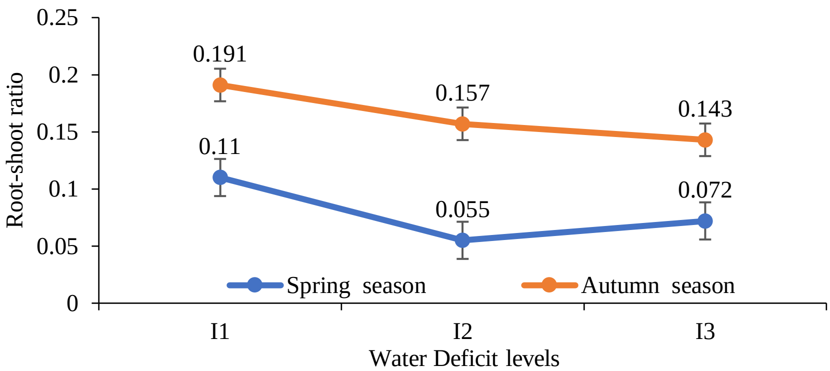

Figure 1. The effect of *water* deficit levels on the R/S in spring- and autumn-grown maize hybrids. For comparison, the L.S.D values for both seasons were 0.036 and 0.188, respectively

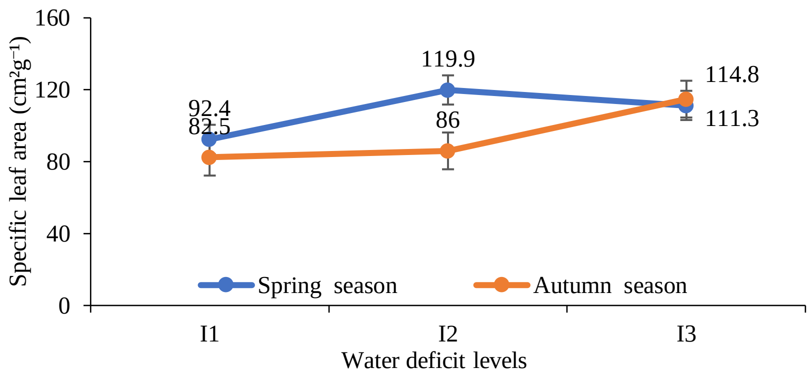

Figure 2. The effect of water deficit levels on the SLA ($cm^2.g^{-1}$) in spring- and autumn-grown maize hybrids. For comparison, the L.S.D values for both seasons were 87.76 and 20.51, respectively

Figure 2. As it can be seen, the SLA of the maize plants were significantly exceeded under the stress levels of irrigation level I_3 compared to the well-irrigated level I_1. The higher SLAs in maize grown at I_2 and I_3 levels in the autumn were due to a shortage in dry matter partitioning to leaves (114.8 $cm^2.g^{-1}$). Conversely, the SLA under I_1 grown maize samples did not exceed (82.5 $cm^2 g^{-1}$; Figure 2).

The study observed significant SLA differences in the combined irrigation scheme levels and responses of maize hybrids in both growing seasons (Figure3 and 4). The SLA generally increased in both seasons from water-deficit levels

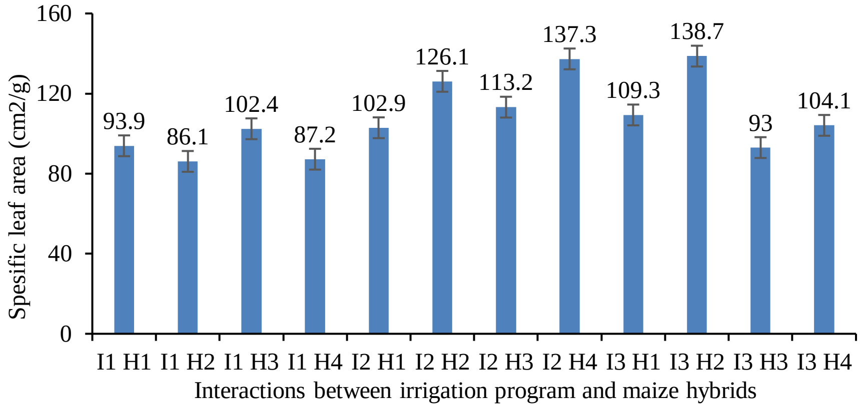

Figure 3. The interaction between the irrigation scheme and maize hybrids for SLA of spring-grown maize hybrids

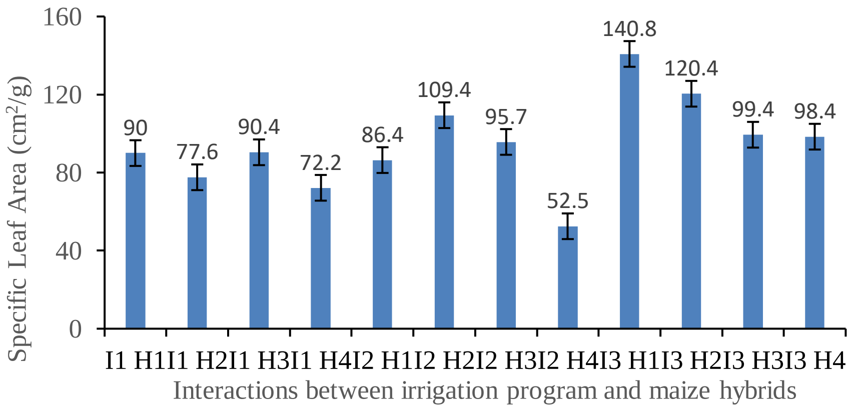

Figure 4. The interaction between irrigation scheme and maize hybrids for SLAs of autumn-grown maize hybrids

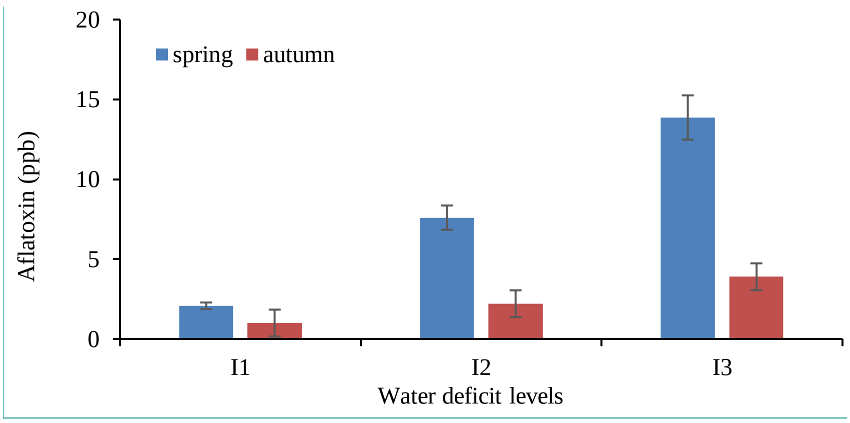

Figure 5. Effect of water deficit levels on aflatoxin contamination in maize hybrids based on L.S.D $_{0.05}$ (3.544, 1.008)

I_1 to I_3. The outcome seen here was consistent with the reduced RCW in maize plant leaves, which directly affected their cell division and leaf expansion. The maximum SLA was noted for maize plants grown under the I_3 water deficit level, whereby the response of H_3 (I_3H_3) was 138.7 cm^2 g^{-1} in spring. Conversely, the I1H2 sample showed the lowest SLA (Figure 3). However, the autumn-grown maize hybrids recorded maximum and minimum SLA for the I_3H_1 (140.38 cm^2 g^{-1}) and I_2H_4 (52.5 cm^2 g^-1) samples. Noteworthy, the significant differences in the SLA values ($p < 0.05$) of the produced maize demonstrate the profound influences of different seasons and irrigation schemes that affect leaf development and plant growth in this study. The varying reduction in leaf water potential and photosynthetic efficiency in the studied maize samples following growth under water shortage might also be affected by genetics. Moreover, the quality of light intensity, which affects the photosynthesis process, tends to manifest in the plant's susceptibility and tolerance to adverse environmental conditions. The same factor (light intensity) might also influence the plant's phenological development [26].

Aflatoxin contamination

The study discovered substantial differences in the varying water deficit levels on the severity of aflatoxin contamination in the resultant seasons. The spring-grown H4 maize hybrids. Figure 5 illustrates the minimum aflatoxin contamination level detected in maize grown under I1 water deficit level (2.08 ppb) for both seasons. In contrast, the highest level of aflatoxin contamination occurred under the I3-grown maize (13.87ppb). This study also detected significant differences in the maize hybrid's responses to aflatoxin contamination in both exhibited the highest aflatoxin contamination level. The H2 and H3 hybrids were also highly susceptible to contamination by 9.83 and 8.71 ppb, respectively (Figure 6). It can be

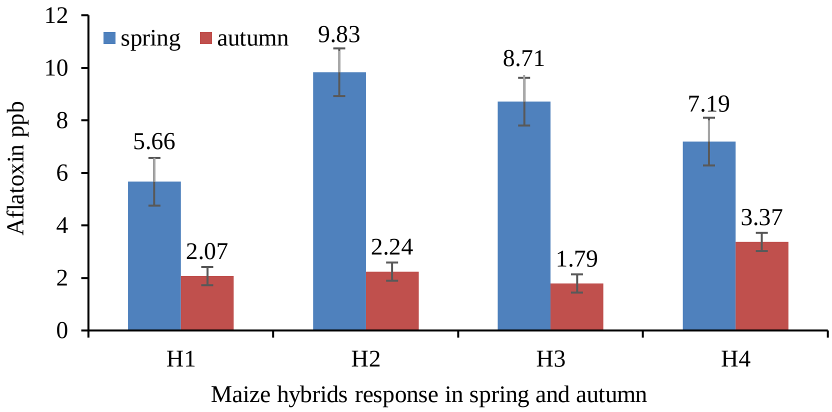

Figure 6. The responses of maize hybrids to aflatoxin contamination in maize hybrids based on L.S.D $_{0.05}$ (7.019, 3.37)

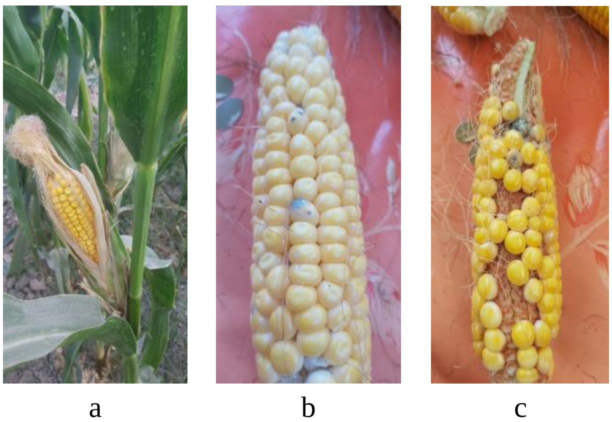

Figure 7. Morphology of maize. (a) Tolerance maize hybrid during the dent stage in the autumn growing season, (b) Ear with contaminated kernels accompanied by Aspergillus flavus in spring, and (c) Contaminated hybrid during the ear formation in spring.

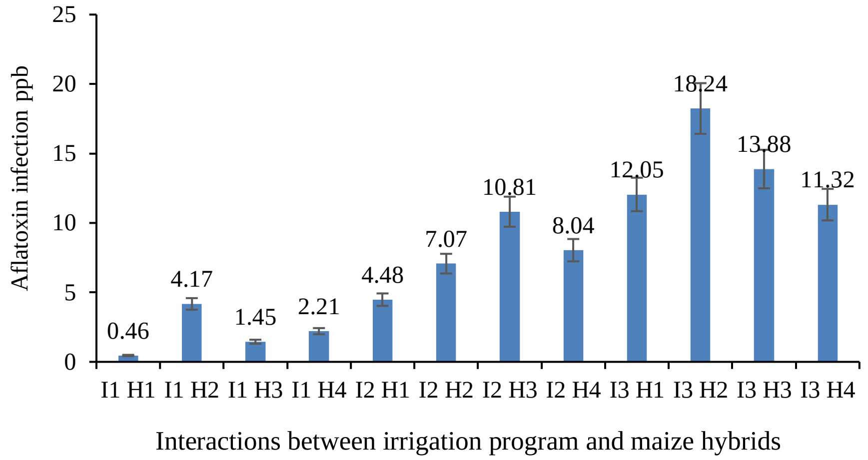

Figure 8. The effect of water deficit levels and severity of aflatoxin contamination in spring-grown maize hybrids

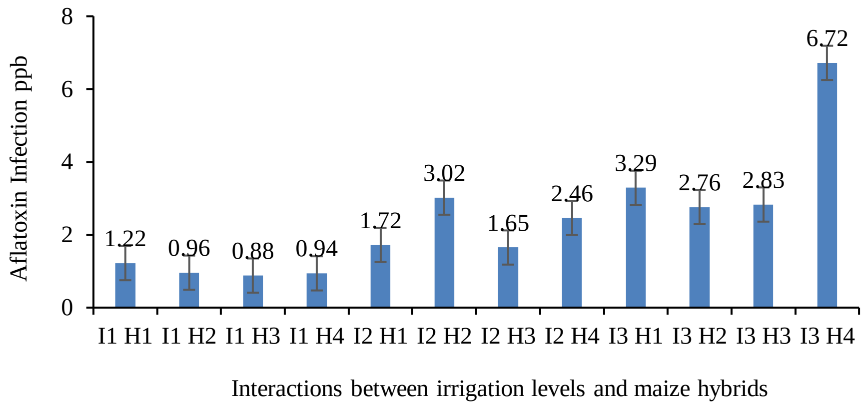

Figure 9. The effect of water deficit levels and severity of aflatoxin contamination in autumn-grown maize hybrids

construed that the higher aflatoxin contamination in spring-grown maize was correlated to the higher daily temperature. Such a condition has been known to increase evapotranspiration in plants, and creates a better breeding condition for Aspergillus flavus, as previously reported by Abbas et al. [21]. Nevertheless, the autumn-grown maize samples were more tolerant toward aflatoxin contamination. This was apparent in their lower contamination levels of 2.24 and 1.79 ppb (Figure 5).

Figure 7 and 8 depict the levels of aflatoxin contamination in maize hybrids grown in both seasons. As it can be seen, the contamination levels in spring-grown maize varied between different water deficit levels and maize hybrids, ranging between 0.46 to 18.24 ppb for the I1H1 and I3H2 treatments, respectively. The maize hybrid H1I1was the least affected (0.46) by aflatoxin contamination. The aflatoxin contamination was the highest for maize samples grown under the I3 water deficit level. The high contamination level in the maize samples was notable from the V5 growing phase to the dent stage post-silking, which is the most critical and sensitive period for aflatoxin contamination [14]. The outcome is seen to correspond well to the water-stress condition due to higher environmental temperatures during these growth periods (Table 1). The aflatoxin contamination level demonstrates the opportunity for infection of maize kernels with Aspergillus flavus. Although the aflatoxin contamination levels were lower than the Food and Drug Association (FDA) limit (20 ppb), the study notes an apparent risk of aflatoxin contamination in these maize hybrids cultivated in semi-arid conditions. This is largely due to water shortage and high temperatures during the plant's reproductive growth stages [12].

Contrariwise, autumn-grown maize fared better and exhibited lower aflatoxin contamination levels at (0.88 to 6.72 ppb) compared to spring-grown ones (0.46 to 18.24 ppb; Figure 8). This was visible in the well-irrigated I1H3 maize hybrids that showed aflatoxin contamination levels between 0.88 to 6.72 ppb. Meanwhile, the autumn-grown I3H4 treated maize hybrids were less susceptible to aflatoxin contamination (6.72 ppb; Figure 9) than the spring-grown ones (11.32) (Figure 8). This was probably due to the lower daily temperatures that the maize hybrids were exposed to during their reproductive stage, where contamination with aflatoxin was critical.

Conclusion

The study demonstrated the impact of water deficit levels on physiological responses, viz. RWC, SLA, and R/S ratio and aflatoxin contamination in maize kernels under I2 and I3 water deficit levels. Although the study found that aflatoxin contamination in the affected maize kernels was below the FDA threshold of 20 ppb, the risk of crop infection by A. flavus and contamination remains prevalent. This is due to water shortage in water-stressed conditions and high daily temperatures, especially in the spring, which are ideal growing conditions for the pathogenic fungus, A. flavus. The I3 water deficit level led to severe aflatoxin contamination in the maize hybrids observed in this study, as the fungus infection extended to the grain-filling phase. This increases the susceptibility of developing higher concentrations of aflatoxin-contaminated maize kernels. Hence, maize hybrids grown in semi-arid zones should be well-irrigated, especially during the critical grain-filling phase, to minimize the severity of fungal infection. Also, cultivation in moderate climatic conditions, namely in autumn, could reduce the levels of aflatoxin contamination in maize kernels.

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