# Verification of the Accuscan gold reader and RIDA smart phone application rapid test kits in detection and quantification of aflatoxin levels in maize from selected regions in Kenya

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Received: 6 May 2022; Accepted: 11 July 2022; Published: 13 October 2022


**RESEARCH ARTICLE**

## Abstract

The study aimed to verify the effectiveness of two detection kits namely, Accuscan gold reader and RIDA total aflatoxin smart phone application in assessing levels of aflatoxins' contamination in maize. Three regions of Kenya were selected namely, Kisumu, Makueni and Kilifi. Confirmatory tests were done using liquid chromatography tandem mass spectrometry (LC-MS/MS). The Accuscan gold reader showed mean aflatoxin levels of 14.52, 5.18, and 3.04 µg/kg and RIDA smart phone application had levels of 26.08, 26.68, and 4.05 µg/kg for Kilifi, Makueni, and Kisumu, respectively. The LC-MS/MS confirmed the presence of AFB1 and AFB2 with AFB1 of 97.5 µg/kg in Makueni and 76.2 µg/kg in Kilifi. Maize from Kilifi and Makueni was contaminated and unfit for human and animal consumption. Also, low rainfall, high temperature and high relative humidity directly increased the levels of aflatoxins, resulting in contamination. Therefore, the detection kits are recommended for use by maize farmers. Confirmatory tests should be done with maize having levels of aflatoxin above 10 µg/kg. Adequate drying and handling of maize and proper storage with good aeration should be ensured.

*Keywords:* Aflatoxins, liquid chromatography; quantification; rapid test kits

## Introduction

Mycotoxins produced by *Aspergillus*, *Penicillium*, and *Fusarium* cause food decomposition during handling and storage, causing heavy economic losses and serious human and animal health issues (Bangar *et al*., 2022). They are found in agricultural products before, during, and after harvest, and also during food processing (Onyedum *et al.,* 2020). The eastern region of Kenya is known to be a hot spot area for aflatoxicosis, a serious illness caused by aflatoxins and related fungal spores. It is a dangerous phenomenon because aflatoxicosis is associated with human mortalities (Obonyo and Salano, 2018). Aflatoxicosis has frequently affected Kenyans, predominantly in the eastern region, due to the consumption of contaminated maize (Kilonzo *et al*., 2014). Aflatoxin contamination is a public health problem all over Kenya affecting human health due to prevalent dietary exposure as maize is a common staple food crop for the majority of the citizens (Yard *et al*., 2013). Also, maize meal is eaten at a higher frequency and in higher amounts suggesting a danger of aflatoxicosis looming large in the country (Mburu, 2021). For this reason, there is a high demand in sub-Saharan Africa for inexpensive and available techniques for on-site detection of aflatoxins for food safety management (Wacoo *et al.,* 2018).

The farming community of most African countries rely mainly on the climate variables of rainfall, temperature, and humidity for crop production. Furthermore, production of mycotoxins is climate-sensitive where weather and climate variables increases high susceptibility to mycotoxin contamination in Africa. One of the continents with high mycotoxin contamination of foods and remains at record high with incidences of liver cancer globally is Africa (Nji *et al*., 2022). In addition, climatic factors influence fungal occurrence, such as high temperature and humidity, influence fungal occurrence, thereby increasing the risk of fungal growth and mycotoxin production. The same climatic factors determine the survival, distribution, and frequency of mycotoxigenic fungi and subsequent toxin accumulation (Daou *et al*., 2021).

There are six forms of aflatoxin: B1, B2, G1, and G2 found in plant-based foods and M1 and M2 found in foods of animal origin which are metabolites of AFB1. The group AFB1 is the most potent form due to its direct link to human liver cancer (Negash, 2018). Also, milk is an important product in Kenya having the largest dairy herd and the highest per capita milk consumption in East Africa. Feeds contaminated with AFB1 are fed to cows, the milk produced contains AFM1, and after consumption of such milk the AFM1 accumulates in the body posing a risk of liver cancer (Sirma *et al*., 2019). In Kenya, milk from retailers and traders in low-income areas and middle -/ high-income areas are up to 50% contaminated with AFM1 with levels exceeding 50 µg/kg (Lindahl *et al*., 2018). High frequencies of $AFM_1$ in yogurt and other traditional products than the recommended tolerable limits were reported in Kenya (De Souza *et al*., 2021). The standard total aflatoxin limit allowed in Kenya in food and feed is 10 µg/kg for the sum of aflatoxins and 5µg/kg for AFB1 (Sirma *et al*., 2018). Failure of strict regulatory implementation of maximum limits has caused widespread exposure to toxins for maize consumers.

Rapid diagnostic techniques are mostly based on immunochemical assays, with ELISA as a key example, dipsticks, flow-through membranes, and Lateral Flow Detection (LFDs) (Wolf and Schweigert, 2018). The antibody and antigen binding association is exploited varying from simple lateral flow immunoassay and ELISA to very sophisticated immunosensors for mycotoxin testing (Turner *et al*., 2015). Examples of rapid test kits from Neogen Company are Veratox, Reveal Q+, and Agriscreen from the R-Biopharm Company, specifically RIDA quick aflatoxin and RIDA screen aflatoxin, among others (Wolf and Schweigert, 2018). Due to the lack of an on field-testing kit at the farm level, aflatoxin contamination is never detected before getting into the food chain. The use of detection kits at the farm level would ensure that maximum allowable limits for aflatoxin levels are not exceeded. The study was done to determine the effectiveness of rapid test kits in assessing levels of aflatoxin contamination in maize in the three regions of Kenya using two rapid test kits, namely, Accuscan gold reader from Neogen company (Lansing USA) model number 9595 and RIDA smart phone application from R-Biopharm Company (Darmstadt, Germany).

## Materials and Methods

### Experimental sites

Makueni, Kilifi and Kisumu were used as experimental sites and climatic details were obtained from Jaetzold *et al*. (2006). Kilifi weather details were from Jaetzold *et al*., (2012) and Kisumu weather details were from Jaetzold *et al*. (2009), as shown in Table 1. The sites used for the experiment are shown on the map of Kenya in Figure 1. The experimental sites of Kilifi had Coastal lowlands zones (CL) 3 and CL4 as the predominant zone, Makueni had lower midland zone (LM) 3, LM4 and upper midland zones had (UM) 4, and Kisumu had UM1, LM2, and LM4. The agro ecological zones determine which crops are grown in the regions.

### Statistical analysis

Data analysis for the experiment was performed using SAS version 8.02 (SAS/STAT software 1999). The analysis of variance (ANOVA) for aflatoxin quantities was done for mean comparison and standard deviations.

**Table 1. Weather description of the study sites.**

| County | Altitude meters above sea level | Minimum temperatures in $^0C$ | Maximum temperatures in $^0C$ | Rainfall in milli meters per year | Agro ecological zones (AEZ) |
|---|---|---|---|---|---|
| Kilifi | 50 | 28.8 | 33.7 | 600 | CL |
| Makueni | 900 | 13.7 | 28 | 850 | LM and UM |
| Kisumu | 1131 | 23.2 | 29.3 | 1388 | LM and UM |

**Key:** CL: Coastal lands zones, LM: Lower midland zones, UM: Upper midland zones.

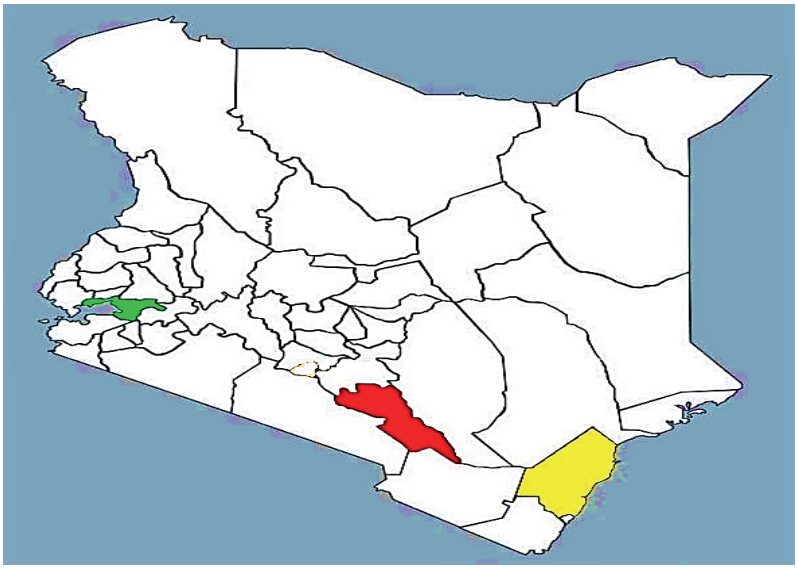



**Figure 1. Map of Kenya showing the study sites.**

Mean separation was done using the least significant difference (LSD) and coefficient of determination with correlation coefficient was used to measure the relationship between two variables of two rapid kits and the LC-MS/MS. The Pearson's correlation coefficient statistical association, between two continuous variables was used.

### Rapid test kits for detection and quantification of aflatoxin levels in maize under field conditions

The experiment comprised two rapid test kits, namely, Accuscan gold reader from Neogen, shown in Figure 2, with the accessories and RIDA smart phone application from R-Biopharm Company with the components displayed in Figure 3.

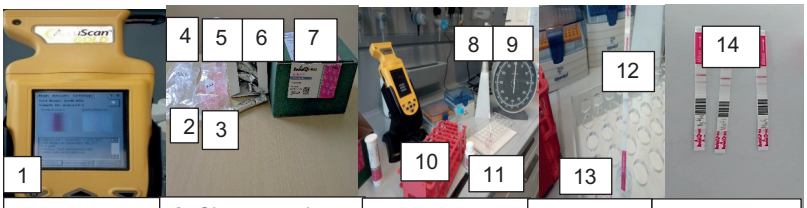
### Sample collection and management

Maize samples were collected from four agro ecological zones in each of the three study sites of Kilifi, Makueni and Kisumu. The samples were labelled accordingly; 45 farms were selected from each county and divided into two. The maize samples had the moisture content (MC) determined using a moisture meter model Draminski ultra sound scanners, samples found having > 13% MC were dried by placing on canvas sheets until all the samples attained ≤ 13 MC. The samples were ground into flour; analysis was done on whole maize flour for total aflatoxin in microgram per kilogram (µg/kg). Each kit had its own set of maize samples which were allocated three test strips each. In the experimental design a complete randomized design (CRD) with three replicates was carried out. When using the two rapid test kits, each sample was analyzed thrice.

### Aflatoxin detection and quantification using Accuscan gold reader platform and reveal Q+MAX

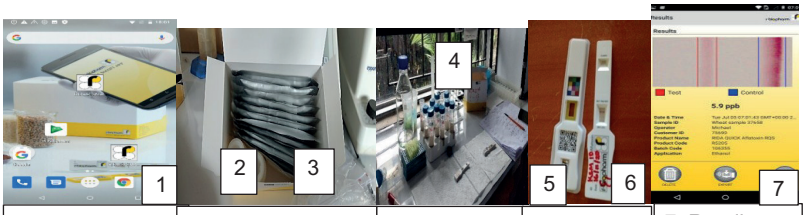
The Neogen machine uses assay principles from Reveal Q+ MAX for aflatoxin quantitative test of a single step lateral flow imunochromatographic assay based on a competitive immunoassay. The Reveal Q+ MAX for aflatoxin testing kit was used in comparison with the AccuScan gold reader. One test kit box includes 25 Reveal Q+ MAX for aflatoxin test strips, 25 red sample dilution cups, 25 clear sample cups, 1bottle of sample diluent and 25 Max 1 aqueous extraction packets.

During analysis, a Whatman # 4 filter paper was used for filtering the flour and Reveal Q+ Max powder contents. In addition, a sample cup rack was used. Two pipettes were used with pipette tips which hold 100 mL together with the 500 mL of distilled water and maize sample filtrate respectively. The methodology adopted from Omara (2019) when using the AccuScan gold reader procedures was followed where 10 g of the maize sample usually in flour form were used. The dehusked maize grains were ground and the flour passed through a 2 mm sieve. The 10 g flour was placed into a plastic bottle of 50 mL




| 1. Accuscan gold reader machine and black cartridge inserted with strip inside. | 2. Clear sample cup<br>3. Red sample cups<br>4. Sample diluent<br>5. Reveal Q+ test strips<br>6. MAX 1aqueous extraction packets<br>7. Test kit box | 8. Pipettes<br>9. Clock timer<br>10. Rack cup holder<br>11. Cartridge adapter | 12. Strip inserted into a cup<br>13. Clear sample cup with diluent | 14. Aflatoxin test strips with readings |
|---|---|---|---|---|

**Figure 2. Accuscan gold reader and accessories from Neogen Company.**




| 1. RIDA smart phone application on a smart phone | 2. Ready to use buffer<br>3. Extraction powder | 4. Flour with buffer mixture on rack | 5. Cover slip<br>6. Labelled strip | 7. Reading on the phone to be recorded |
|---|---|---|---|---|

**Figure 3. Rida smart phone application and accessories from R-Biopharm Company.**

capacity. To the same bottle, one Reveal Q+ Max packet in powder form and 50 mL of distilled water were added. The contents were shaken vigorously using a shaker or hand for 3 min.

The mixture was allowed to settle and filtered with a Whatman # 4 filter paper in a beaker. After the maize sample had been filtered, a minimum of 3 mL of filtrate was collected. In the analysis of aflatoxin when the level was above 50 µg/kg, a dilution was done. A sample cup rack was used for holding the cup and 10 mL of diluent was pipetted into the red sample cup and 100 mL of maize sample filtrate was added. The contents were mixed using a pipette six times. After mixing, 100 mL of the mixture was pipetted and transferred to a clear cup.

A new Reveal Q+MAX for Aflatoxin test strip was placed inside the cup ensuring that the test strip is in contact with the liquid and the timer was set for 6 min. The Reveal Q+ test strip was then fully inserted into the R-labelled cartridge adapter with the sample end first and result the facing out. The cartridge with test strip upside-down was then inserted into the Accuscan gold reader. The results were displayed on the reader and stored in the computed system. When the results displayed are greater than 50 µg/kg, the filtrate was diluted at 6 dilution factor that is 500 mL distilled water and 100 mL filtrate. The date and time when the test was done was also recorded.

### Methodology for RIDA smart application

For this application, the test kit box had its own accessories different from the Accuscan gold reader. The smart phone application starts with a registration with a username and password and requires a voucher from R-Biopharm, with a code only acquired once that is typed into the application. The application on the smart phone allows the user to the main menu where the country is defined followed by description of the workflow mode and frequency validation while using the Internet. The smart phone application form uses a QR code (Quick Response Code) with a RIDA smart phone application color sheet. The QR code encodes basic data like test name and lot number but also links to specific application information of the test lot.

A test strip was used by inserting in the sample matrix contained in a clear bottle; it was then placed in a cartridge holder and covered by a strip. The measurement button in the main menu was selected to start measurements. The sample identity was done for example maize

sample number 5, the customer ID was identified that is the name of the person using the application. The QR code on a cover strip was scanned; an application was chosen and confirmed by pressing the next button. The test strip was scanned and placed within black markings. The camera button was then pressed and auto-focused to capture an image and measurement was displayed and recorded automatically. The date and time when the test was done was also recorded.

The difference between the Rida smart phone application and Accuscan gold reader was the sample diluent which had to be reconstituted at one part buffer and nine parts distilled water. Preparation of ready- to- use extraction buffer was done, involving taking 10 mL buffer and 90 mL distilled water and homogenizing for 5 min. For 10 g of ground flour, 20 mL of reconstituted buffer was added. The mixture was then centrifuged for 3 min to obtain a particle solution, where 100 µL (0.1 mL) was pipetted and applied on the strip and left to stand for 5 min. The reading line appeared and the camera was placed on top of the strip to take a picture, then the aflatoxin quantities were displayed on the screen. Whenever the reading was above 50 µg/kg more dilution was done with 0.1 mL of extract solution and 70 mL of reconstituted buffer.

### Comparative analysis of maize samples with rapid tests to LC-MS/MS

The two sets of maize samples used in analysis with the two rapid test kits were also analyzed for confirmatory tests with liquid chromatography-tandem mass spectrometry (LC-MS/MS) following the methodology from Ouakhssase *et al.* (2019). This was done as a confirmatory test to correlate the values of the kits for the determination of accuracy. The analysis was done at Kephis analytical laboratory, the LC-MS/MS equipment name was Agilent LC-MS/MS, Model number 6460, serial number DE16437004, and code ACL- LC-MS/MS_02. Chromatographic conditions; chrome type TIC, label TIC, offset 0 and Y-range 10000000. The MS/MS parameters; source parameters' gas temperature positive value was 325 °C, negative value 325, gas flow (L/min) positive 10, negative 10, Nebulizer (psi) positive 45, negative 45, capillary (V) positive 4000, negative 4000. Injector parameters; in the acquisition method, the draw speed was 200 µL/min, eject speed was 200 µL/min, draw position offset 0.0 min, injection mode was the standard injection, injection volume 10.00 µL, high throughput. Stop time as pump/no limit.

Other attributes of the analytical method; chromatographic separation of the targeted analytes was performed using a ZORBAX RRHD Eclipse plus C18 Capillary column with dimensions of 2.1 × 150 mm, 1.8 µm (part number 959759-902) installed on an Agilent 1290 Infinity II LC system with an Agilent 1290 Infinity II high-speed pump (G4220A), Agilent 1290 Infinity II auto sampler (G4226A) and Agilent 1290 Infinity II thermostatted column Compartment (G1316C): The LC conditions used were as follows: column temperature was set at 40 °C, injection volume was 10 µL, mobile phase A was 5 mM ammonium formate in water with 0.1% formic acid and mobile phase B was 5 mM ammonium formate in Acetonitrile with 0.1% formic acid. The mobile phase flow rate was 0.3 mL/min. The gradient elution program was 5% B at 0.8 min, 70% B at 2 min, 60% B at 4.20 min, 60% B at 7min, 95% B at 8 min, and post-run 6 min.

## Results

The Accuscan gold reader showed mean aflatoxin levels in µg/kg of 14.52, 5.18, and 3.04 in µg/kg for Kilifi, Makueni, and Kisumu, respectively. The LC-MS/MS means were 9.28, 2.63, and 2.0 for the same areas. Both means from the Accuscan gold reader and LC-MS/MS were added and divided by two to give the overall average of 12.0, 3.91, and 2.53 in µg/kg as shown on the last row in Table 2. RIDA smart phone application gave total aflatoxin levels in µg/kg of 26.68, 26.09, and 4.05 for Kilifi, Makueni and Kisumu, respectively. The LC-MS/MS means were 3.43, 13.17, and 2.01. The average of two means for RIDA smart phone application and LC-MS/MS were also added to give 15.06, 19.63, and 3.01 µg/kg respectively as the overall values, as shown in the last row of Table 3.

The ranges of aflatoxin levels were 2 - 90.7 µg/kg when using Accuscan gold reader and 4 -198.9 µg/kg when using the RIDA smart phone application. The first set of maize samples showed significant differences from the three sites of Kilifi, Makueni, and Kisumu analyzed by using the Accuscan gold reader for total aflatoxins shown in Table 2. The RIDA smart phone application had no significant differences for samples in Kilifi and Makueni when using the second set of maize samples, while Makueni and Kilifi showed differences to Kisumu, as shown in Table 3.

### Confirmatory tests

Confirmatory tests were done and six samples tested positive to the (LC-MS/MS) analysis when comparing to the Accuscan gold reader and one sample showed results closer to the LC-MS/MS results, as shown in Table 2. The maize samples analyzed using the RIDA smart phone application had five samples testing positive. When the same maize were tested using the LC-MS/MS for verification only one sample gave results closer to the

LC-MS MS. The results for two samples, one each from the Accuscan gold reader and RIDA smart phone application were almost similar to each other, as shown in Tables 3 and 4. The tests using LC-MS/MS identified two types of aflatoxins, AFB1 and AFB2, which were added up to calculate total aflatoxins.

**Table 2. Total aflatoxin (µg/kg) means for Accuscan gold reader and liquid chromatography tandem mass spectrometry (LC-MS/MS).**

| Accuscan gold reader means | | | | |
|---|---|---|---|---|
| **County** | **Accuscan gold reader Means** | **LC-MS/MS** | **SD Accuscan** | **SD LC-MS/MS** |
| Kilifi | 14.52[a] | 9.28[a] | 7.13 | 6.92 |
| Makueni | 5.18[b] | 2.63[b] | 0.14 | 0.01 |
| Kisumu | 3.04[c] | 2.01[b] | 2.23 | 0.64 |
| LSD | – | 2.05 | 2.45 | – |
| $\alpha$ = 0.05, CV = 28.4, $R^2$ = 0.12 | | | | |

SD: Standard deviation, LSD: Least significant difference, CV: Coefficient of variation, Letter used in mean separation: a, b, and c showing that means with the same letter are not significantly different along the columns. Means with different letters are significantly different.

**Table 3. Total aflatoxin (µg/kg) means for RIDA smart phone application and liquid chromatography tandem mass spectrometry (LC-MS/MS).**

| RIDA smart phone application means | | | | |
|---|---|---|---|---|
| **County** | **RIDA Means** | **LC-MS/MS** | **SD RIDA** | **SD LC-MS/MS** |
| Kilifi | 26.68[a] | 3.43[b] | 19.31 | 0.93 |
| Makueni | 26.09[a] | 13.17[a] | 0.035 | 0.01 |
| Kisumu | 4.05[b] | 2.01[b] | 14.79 | 9.82 |
| LSD 2.05 | – | 2.45 | 21.27 | – |
| $\alpha$ = 0.05, CV= 27.9, $R^2$ = 0.09 | | | | |

Letter used in mean separation: a, b, and c showing that means with the same letter are not significantly different along the columns. Means with different letters are significantly different.

The content of AFB1 in two maize samples occurred in high quantities of 20 times above the regulatory limit of 5µg/kg defined by the Kenya Bureau of Standards (KEBS) (Tables 4 and 5). The incidence of AFB1 was high in all samples tested positive by LC-MS/MS (Tables 4 and 5). When quantification for total aflatoxins (AFB1 and AFB2) was done usig LC-MS/MS, Makueni had the highest level of AFB1 found in Mbumbuni (UM4) at 97.5 µg/kg. In samples from Kilifi in Mtepeni (CL4), the content was 76.2 µg/kg for AFB1. Samples from Mbumbuni in Makueni and Mtepeni in Kilifi showed higher levels of aflatoxin in maize compared to the other regions (Figures 4 and 5). Samples from Kisumu had low aflatoxin levels within the allowable limits of 10 µg/kg with the AEZ of LM and UM shown in Table 1. The rapid test kits displayed results with no significant differences to the LC-MS/MS.

### Relationship between LC-MS/MS and Accuscan gold reader for aflatoxin detection and quantification

Coefficient of determination ($R^2$) showed a relationship (85.6%) between LC-MS/MS and Accuscan gold, as shown by the equation. The coefficient of correlation (R) for LC-MS/MS and Accuscan gold reader was 0.925 (R), as depicted by the equation. The graph of the correlation equation y = 0.8381x - 1.7107, with y for LC-MS/MS and x for Accuscan gold reader is shown in Figure 6.

Coefficient of determination ($R^2$) displayed a relationship of 31.88% between LC-MS/MS as a confirmatory test and RIDA smart phone application. The coefficient of correlation (R) for LC-MS/MS and RIDA smart phone application was 0.565 (R). The graph of the correlation equation y = 0.2313x + 1.8266 described the relationship between

**Table 4. Total aflatoxin, AFB1, and AFB2 levels detected using LC-MS/MS and Accuscan gold reader.**

| County | AEZ | Mean total aflatoxin | LC-MS/MS AFB1 | LC-MS/MS AFB2 | Mean total aflatoxins LC-MS/MS |
|---|---|---|---|---|---|
| Kilifi | CL3 | 14.8[d] | 2.1 | 0 | 2.1[c] |
| | CL3 | 13.2[d] | 2.2 | 0 | 2.2[c] |
| | CL4 | 17.7[c] | 4.2 | 0 | 4.2[c] |
| | CL4 | 90.7[a] | 76.2 | 9.4 | 85.6[a] |
| Makueni | LM4 | 3.1[e] | 4.7 | 5.0 | 9.62[b] |
| | UM4 | 30.2[b] | 2 | 0 | 2[c] |
| LSD | – | 2.05 | – | – | 2.45 |
| $\alpha$ = 0.05 | | | | | |

AEZ: Agro Ecological Zones, Letter used in mean separation: a, b, and c showing that means with the same letter are not significantly different along the columns. Means with different letters are significantly different.

**Table 5. Total aflatoxin, AFB1, and AFB2 levels detected using LC-MS/MS and RIDA smart phone application.**

| County | AEZ | Mean total aflatoxin | LC-MS/MS AFB1 | LC-MS/MS AFB2 | Mean total aflatoxins LC-MS/MS |
|---|---|---|---|---|---|
| Kilifi | CL3 | 30.2[d] | 6.7 | 0 | 6.7[d] |
| | CL3 | 9.7[e] | 2.9 | 0 | 2.9[e] |
| | CL3 | 198.9[a] | 10.7 | 0 | 10.7[c] |
| Makueni | UM4 | 105[b] | 9.8 | 5.1 | 14.9[b] |
| | UM4 | 123.9[c] | 97.5 | 3.3 | 100.8[a] |
| LSD | – | 2.05 | – | – | 2.45 |
| $\alpha = 0.05$ | | | | | |

Letter used in mean separation: a, b, and c showing that means with the same letter are not significantly different along the columns. Means with different letters are significantly different.

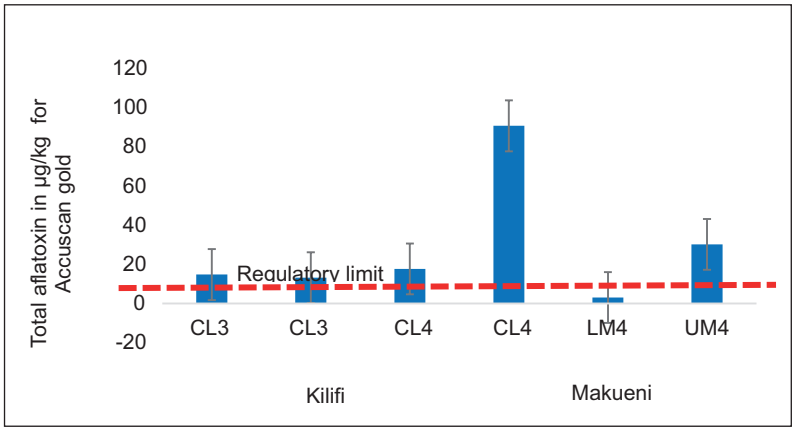



**Figure 4. Total aflatoxin in µg/kg in Kilifi and Makueni.**

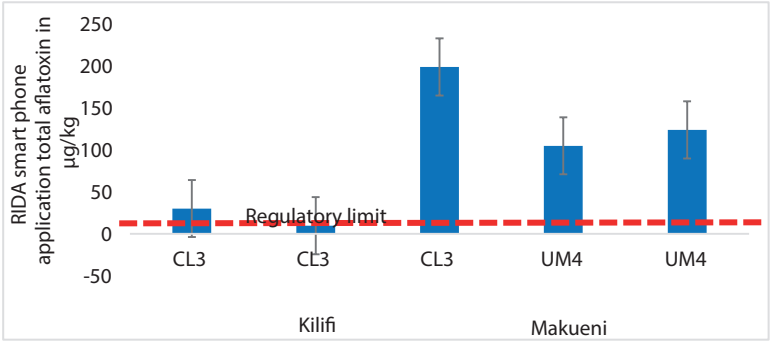



**Figure 5. Total aflatoxin in µg/kg in Kilifi and Makueni.**

both methods (Figure 7). In the equation, y was the LC-MS/MS and x was the RIDA smart phone application.

## Climatic factors influencing aflatoxins' prevalence and intensity

The weather pattern for the study period of 12 months of 2020 was collected for rainfall, temperature and relative humidity and averages done. The rainfall in millimeters for the three study sites during the period when the experiment was done had Kisumu with 362.77 mm, Makueni 56.81 mm, and Kilifi 105.73 mm. The minimum and maximum temperatures in degrees Celsius (°C) for Kisumu were 18.18 and 28.53, Makueni 17.42 and 29.1, and Kilifi 24.18, and 30.4. The maximum and minimum relative humidity for Kisumu were 53.58 and 70.58, Makueni 49.42 and 80.25, and Kilifi 70.58 and 80.25 (Table 6).

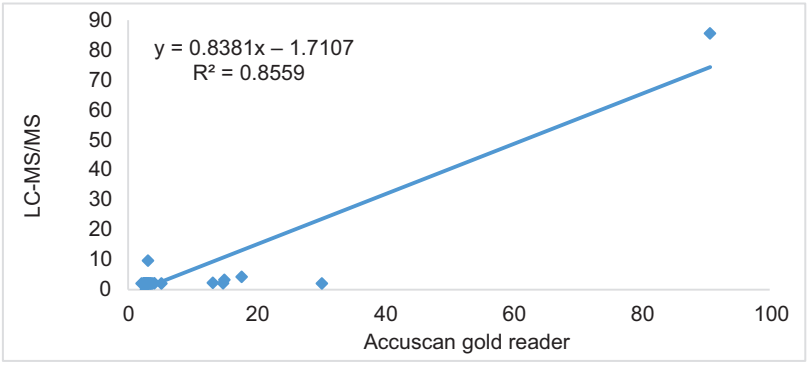



**Figure 6. Relationship between LC-MS/MS and Accuscan gold reader.**

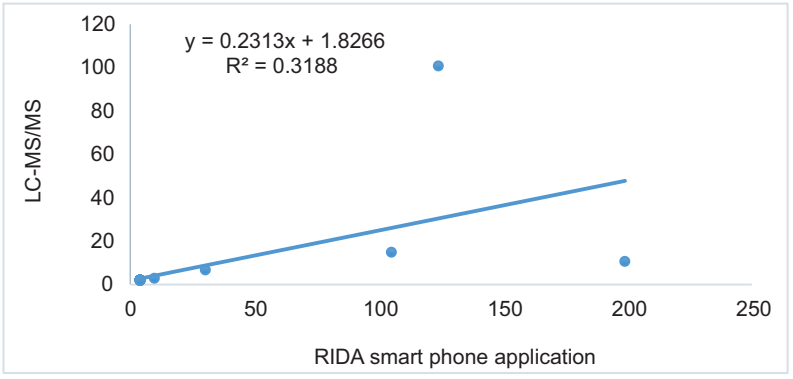



**Figure 7. Relationship between LC-MS/MS and RIDA smart phone application.**

**Table 6. Weather data from the study sites during 2020.**

| Station | Rainfall mm | Temperature minimum | Temperature maximum | Relative Humidity minimum | Relative Humidity maximum |
|---|---|---|---|---|---|
| Kisumu | 362.77 | 18.18 | 28.53 | 53.58 | 70.58 |
| Makueni | 56.81 | 17.42 | 29.1 | 49.42 | 80.25 |
| Kilifi | 105.73 | 24.18 | 30.4 | 70.58 | 80.25 |

## Discussion

In the three study sites, total aflatoxin was detected and quantified using the two rapid test kits. With the Accuscan gold reader, aflatoxin levels from Kilifi were exceeding the regulatory limits of 10 µg/kg. In samples from Makueni 5.2 µg/kg was determined and in samples from Kisumu it was 3.0 µg/kg. The quantities in samples from Kilifi and Makueni were above the maximum allowable limits. This agreed with Yard *et al.* (2013), who reported that total aflatoxin was the highest in eastern and coastal Kenya and the lowest in Nyanza and Rift valley. In the two rapid test kits used in the detection and quantification of aflatoxins, the Accuscan gold reader gave results closer to the LC-MS/MS as compared to the RIDA smart phone application.

This demonstrates that aflatoxin contamination levels in maize from Kilifi and Makueni are exposing the consumers to health risks. Omara *et al.* (2021) reported of wide spread aflatoxin contamination in Kenya further supporting findings of this study. In the detection and quantification of aflatoxins, liquid chromatography was the main technique used in mycotoxin analysis (Turner *et al.*, 2015). Therefore, in the present study for the detection and quantification of aflatoxins, a coefficient of correlation (R) for LC-MS/MS and Accuscan gold reader of 0.925 was found showing a perfect relationship. The 0.56 coefficient of correlation (R) for LC-MS/MS and RIDA smart phone application presented an average relationship.

According to Pumpa *et al.* (2021), the Rida smart phone application is highly suitable for an inexpensive, quick,

and precise quantitative determination of mycotoxins in agricultural foodstuffs such as aflatoxins in corn. It is also proven to provide reliable and precise results at any time. In the study, the RIDA smart phone application correlation coefficient was average in aflatoxin analysis. In Cvak *et al*. (2021), it is described that during analysis when the maize flour is not well refined during grinding, spot and cross contamination while handling maize samples, analysing samples with temperature of above 30°centigrade, when using lateral flow disks results are affected. Shaking of samples during analysis if not well mixed in less than the stipulated time from the manufactures instructions, origin of samples with nonhomogeneous samples, gave incorrect and invalid results. The presence of different matrices and variable origins of the maize samples gave incorrect and invalid results. According to Luis and Kemerait (2015) fluctuating and high temperatures are normal in tropical environments. Storage of rapid kits test' strips at a high temperature of over 34° centigrade combined with long periods starting 35 to 53 weeks, gave invalid and incorrect results (no bands formed). In addition, the test strips were sensitive to humidity. Humid reaction strips influence the test results. Similarly, test strips have expiry dates and should be kept under the manufacturer recommended storage temperatures.

The validation of Reveal Q+ MAX for aflatoxin testing with Accuscan gold reader was done by Le *et al.* (2019) who confirmed that the machine was adequate in detection and quantification of mycotoxins. Furthermore, the Reveal Q+ MAX for aflatoxin testing offered the advantages of minimal labor, bench space and hardware requirements, disposable materials, and rapid results within 6 min following sample extraction. This was confirmed with the perfect relationship to the LC-MS/MS at 0.925. A study was done by Nyangi *et al.* (2016) quantifying total aflatoxin using enzyme-linked immunosorbent assay (Reveal AccuScan® Neogen, USA) and the results were confirmed using LC-MS/MS. This demonstrated the fact that rapid kits are used alongside gold criterion standards for confirmatory tests. In addition, chromatography is the predominant methodology normally used in food and feed for mycotoxin analysis all over the world (Agriopoulou *et al*., 2020). Hence LC-MS/MS has been used as the standard technique in this study. The LC-MS/MS detected the presence of single aflatoxin types as AFB1 and AFB2, which were added to make total aflatoxins. Khaneghah *et al*. (2019) confirm that liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most sensitive, precise, and accurate technique in mycotoxin detection and quantification.

The Kenyan regulatory limits for total aflatoxins AFB1, AFB2, AFG1, and AFG2 are 10 µg/kg and 5 µg/kg for only AFB1 (Sirma *et al*., 2018). Samples from Makueni had AFB1 levels of 97.47 µg/kg which is nearly 20 times the maximum allowable limits. Eastern Kenya has been reported to be a hot spot for aflatoxin contamination in Kenya due to conducive conditions for the establishment of toxigenic *Aspergillus* as reported by Birgen *et al*. (2020). Luis and Kemerait (2015) confirm that even with the availability of several mechanical and chemical methods for aflatoxin detection, most are not accessible to many developing countries. When the kits were demonstrated to the farmers the response was that the detection and quantification had never reached the household and farm levels. The kits were readily available in colleges and research institutes and farmers had never seen them before. Rapid test kits for total aflatoxins are very straight forward and fast and the total analysis time including sample preparation are generally lower than 10 min. Lattanzio *et al*. (2019) affirm that rapid tests can be regarded as a reliable tool for food safety testing. The cost of buying equipment such as LC-MS/MS and HPLC is quite high and requires trained scientific staff to operate. The costs of analyzing one sample of maize using rapid test kits is 10 times lower than for LC-MS/MS and HPLC which requires ensuring of proper instrument management (Wolf and Schweigert, 2018). Rapid test kits only use color change and the implementation is fast during detection and quantification for aflatoxins.

The climatic patterns of rainfall, temperature, and percent relative humidity determined the levels of total aflatoxin occurrence. Therefore, the weather in the three study sites of Kisumu, Makueni, and Kilifi had a high percentage of maximum relative humidity and temperatures which according to Muga *et al.* (2019) influence the growth of fungi and aggravates aflatoxin contamination of maize. Likewise, in Temba *et al.* (2021), low rainfall combined with high temperatures increase the chances of aflatoxin contamination in maize which proves the case in the study sites. Equally, in Keller *et al*. (2022), the evolving climatic conditions in sub - Saharan Africa, Kenya included, are conducive for aflatoxin production. As stated by Kangethe *et al.* (2017), Nandi and Makueni are hot spot regions for aflatoxin contamination in maize and fall in the same agro ecological zones of lower humid highlands to upper midlands, and upper high land zones (LH2, LH3, LM4, LH5), (UM3 UM4). In the study, the same zones were reporting the highest levels of aflatoxin occurrences.

## Conclusion

Aflatoxin contamination is a common problem in Kilifi and Makueni at levels above the regulatory limits of 10 µg/kg defined by KEBS and low values in Kisumu within the limits of below 4 µg/kg. The AFB1 type of aflatoxin in samples from Kilifi and Makueni occurred in extremely high levels in comparison to the regulatory limit of 5 µg/kg confirming that it is dangerous and very harmful to

humans and animals when maize with such toxic levels is consumed. The two rapid test kits Accuscan gold reader and RIDA smartphone application are appropriate in total aflatoxin testing on maize grains. When using the RIDA smart phone application ensure adequate and proper handling of samples and storage of test strips. The kits proved the effectiveness in total aflatoxin quantification; they are easy to use and cheap and should be recommended for routine total aflatoxin quantification. In cases where levels are above 10 µg/kg, confirmatory tests should be done. The content of aflatoxins in maize grains from Kilifi and Makueni should always be quantified routinely because of AFB1 occurrence in high quantities. Awareness creation to the farmers in high-risk areas of Kilifi and Makueni should be done regularly. The environmental factors of high temperatures and relative humidity increased the chances of aflatoxin contamination of maize in Makueni and Kilifi.

## Recommendation

Aflatoxin testing in maize should be done using the rapid test kits to avoid consumption of contaminated maize in the high-risk areas of Kilifi and Makueni. Likewise, maize grains should always be quantified routinely because of AFB1 presence in high quantities before being allowed to move through the food chain. Maize farmers from Kilifi and Makueni should be supported fully to handle the maize grains from harvesting, drying and storage for food safety. Awareness creation to the farmers in high-risk areas will enable the application of good practices.

## Acknowledgement

This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) based on the decision of the Parliament of the Federal Republic of Germany through the Federal Office for Agriculture and Food (BLE). Beatrice Tenge acknowledges the Max Rubner-Institut in Germany for providing the test kits used in the studies, the Kenya Agricultural Research Institute for providing all the logistical support, and the University of Nairobi for all the support.

## Conflict of Interest

The authors declare no conflict of interest.

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