Evaluation of performance of metal oxide electronic nose for detection of aflatoxin in artificially and naturally contaminated maize

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Abstract

Aflatoxins are of great concern for food safety and security due to their impact on human health and the agriculture economy in developing countries. There is therefore a need for rapid, cost-effective, portable and easy to operate diagnostic techniques for their detection that can be deployed in the field to facilitate removal of contaminated lots. This study aimed to evaluate the potential use of a field portable metal oxide sensors based electronic nose to detect aflatoxin contamination in Kenyan maize varieties that were artificially and naturally infected with *Aspergillus flavus.* Mutual information was used to select features from the electronic nose sensor signals for classification of the samples. The effectiveness of selected features to discriminate between the different classes of samples was evaluated by support vector machines and *k*-nearest neighbour with leave-one-out cross-validation. External validation was also conducted by analysing samples naturally contaminated with *A. flavus* using the classification model generated with samples that had been artificially inoculated with the aflatoxigenic *A. flavus.* Cross-validated classification accuracies ranged from 72 % to 88 % for maize samples artificially inoculated with *A. flavus* and 61 % to 86 % for samples naturally infected with *A. flavus*. Classification accuracies achieved with external validation for maize samples naturally contaminated with aflatoxins ranged from 58 % to 78 % and were relatively consistent with accuracies obtained from internal validation. Results suggest that the electronic nose could be a promising cost-effective screening method to detect aflatoxin contamination in maize.

Key words: *Zea mays*, *Aspergillus flavus,* mycotoxins, electronic noses, volatile organic compounds, mutual information.

1.0 Introduction

Maize (*Zea mays*), the main staple food for much of the population in developing countries, is highly susceptible to contamination with aflatoxins, produced mainly by *Aspergillus flavus* [1]. Aflatoxins, pose a great threat for agriculture, trade and human health in developing countries as a result of economic losses from reduced grain quality, loss of animal productivity and reduced accessibility to international markets [2]. Management of aflatoxin remains problematic, particularly in developing countries where production and storage conditions favour contamination, coupled with a lack of well-established regulatory systems to frequently monitor food samples prior to trade or human consumption [3]. Additionally, contamination is not often evident to the farmer and hence requires robust sampling and analysis technologies [4]. Electronic nose can potentially be deployed as rapid, non-invasive, cost-effective and portable systems for detection of mycotoxin contamination in cereals to provide real time monitoring data to facilitate removal of contaminated lots [5]. Electronic nose detection of mycotoxin is based on capability to detect changes in composition volatile organic compounds (VOCs) in the sample as result of fungal infection [6].

Capability of electronic nose to discriminate between non-infected samples and samples infected with different species or strains of mycotoxigenic fungi in various cereals has been documented [7-14]. Most of the studies have been *in vitro,* with the fungi inoculated on artificial media or sterilised grain. However, biosynthesis of volatile organic compounds is strongly dependant on the environmental conditions of growth. Significant variations in volatile profiles have been observed when mycotoxigenic fungi are grown on different substrates and in different environments [15-17]. Changes in volatile profiles have also been observed between different genotypes of wheat and maize infected with the same mycotoxigenic fungal species [18, 19]. In view of the various factors influencing the production of volatile compounds by toxigenic fungi on grains, deployment of the electronic nose as a diagnostic technique for detection of aflatoxin contamination is contingent on validating its capability to detect contamination in inoculated and, critically, naturally infected maize samples of different maize varieties. The objective of the current study was to evaluate the potential of a field portable metal oxide electronic nose (DiagNose) for the detection of volatiles associated with Kenyan maize varieties artificially and naturally contaminated with aflatoxins.

2.0 Materials and Methods

2.1 Artificial inoculation of maize with *Aspergillus flavus*

Aflatoxigenic (121365) and non- aflatoxigenic *A. flavus* (3VM787) isolates were obtained from the strain collection at the University of Nairobi School of Biological Sciences. They had previously been isolated from maize and soil from farms and rural households in the Makueni and Nandi counties of Kenya, as described by Okoth, Nyongesa [20]. Aflatoxigenic status of the isolates was confirmed under UV light at 350 nm and by direct competitive Enzyme-linked immunosorbent assay (ELISA) as described in Machungo, Berna [21]. Maize cobs at the kernel dent stage of two Kenyan maize varieties, Duma 43 and Pioneer were used for inoculation with *A. flavus* as described in Machungo, Berna [21]. Treatments included contaminating maize cobs with aflatoxigenic and non aflatoxigenic *A. flavus* at two, six and ten incisions per cob (10 cobs per incision, $n = 30$), to achieve different aflatoxin concentration levels. Cobs inoculated with 2 % v/v Tween 20 at two, six and ten incisions served as controls (10 cobs per incision, $n = 30$). The samples were used for evaluation of performance of the DiagNose through a number of pair wise comparisons; control vs all *A. flavus* infected maize (30 vs 60, $n = 90$), control vs aflatoxigenic *A. flavus* infected maize (30 vs 30, $n = 60$), control vs non-aflatoxigenic *A. flavus* infected maize (30 vs 30, $n = 60$) and non-aflatoxigenic vs aflatoxigenic infected maize (30 vs 30, $n = 60$).

2.2 Collection of maize samples naturally infected with *A. flavus*

Maize samples potentially contaminated with aflatoxins under field conditions were collected from farms in the Bura Irrigation Scheme in Tana River County and Kaiti in Makueni County, Kenya. The two sites were selected on the basis of past records that they have high prevalence of aflatoxin contamination, and outbreaks of aflatoxicosis [22-24]. Additional samples were provided by Bioscience eastern and central Africa (BecA- ILRI Hub) and were collected from market centres in Meru County. A total of 195 maize samples comprising three varieties (Pioneer, Duma 43, and DH04) and market samples (mixture of unknown varieties) were collected as described in the **Supplementary materials**. The samples were used for evaluation of performance of the DiagNose through pair wise comparison of uncontaminated vs contaminated maize.The presence and concentration of aflatoxin in collected samples was determined by direct competitive ELISA (Agra Quant® total aflatoxin assay 1/20 Romer labs. Inc., Union, MO, USA) as per manufacturer protocol.

2.3 Electronic nose analysis

The head space volatile profiles of maize experimentally inoculated or naturally contaminated with *A. flavus* as well as the control treatments were analysed with a field portable electronic nose: DiagNose (C-it, The Netherlands) equipped with twelve *n* type metal oxide sensors with thermocycling where the sensor surface temperature is varied over 32 steps following a quasi-sinusoidal signal, between temperatures of 260 ^{o}C and 340 ^{o}C. The sensor array comprises of six types doped tin oxide (SnO_2) sensors and one type undoped tungsten oxide (WO_3) sensors as summarized in **Table 1.**

Table 1: Sensor array for DiagNose

Sensor type	**Doping**	**Sensor ID**
SnO_2-Pd	Palladium	S1, S2, S3
SnO_2-Pt	Platinum	S4, S8, S12
SnO_2-Cu	Copper	S5
WO_3	Undopped	S6, S7, S9
*Experimental sensor ((Extype 1)	-	S10
SnO_2-Ag	Silver	S11

* Composition undisclosed by the manufacturer

Volatiles were extracted as described in Machungo, Berna [21]. In brief, 1.65 g maize flour in a 10 mL glass vials (Supelco, Bellefonte, PA) fitted with a silicon/teflon magnetic auto sampler vial cap (Agilent Technologies, Australia) were incubated in a block heater for 30 min at 35 ^{o}C to allow the release of volatiles into the sample headspace. After incubation, the headspace was carried to and from the electronic nose chamber via Teflon tubing at a flow rate of 40 mL/ min. Headspace was sampled for five minutes.

2.4 Data analysis

Data pre-processing, feature selection, cross-validation and classification were conducted as described in Machungo, Berna [21] and as detailed in the **Supplementary materials**. In brief, electronic nose raw data for different samples analysed within the same day was first normalised by their mean and standard deviation (i.e. z-scored): $(x-\mu)/\sigma$, where x is a data

point, μ is the mean of all data for that sensor that day, and σ is the standard deviation of all data for that sensor. Mutual information (MI) was used for selection of features for classification of the samples. The MI for individual features and respective class comparison was calculated to determine sensors with discriminating information related to the different sample classes. The first 100 features with the most information on the difference between the sample classes were selected. Effectiveness of the selected features to discriminate between the different sample classes was evaluated by two classifiers; support vector machines (SVM linear and radial functions) and *k*- nearest neighbour (KNN) with different values of *k* (*k* = 1, 3, 5, 7, 9, 11, 13) with leave-one-out cross-validation. An accuracy of 95 % was deemed satisfactory for classification. Classification accuracy rates below the significance level value are considered non-significant. The naive classification level, which represents classification rates expected by chance, was also determined. The number and type of misclassified samples (false positive and false negative) were investigated from the classifier that achieved the highest classification rate.

2.5 External validation

The potential for DiagNose to detect aflatoxin contamination in maize was externally validated using data from maize samples artificially infected with aflatoxigenic *A. flavus* as a training set and data from maize samples naturally infected with *A. flavus* as a validation set. This was evaluated for all inoculated samples vs all naturally infected samples, inoculated samples vs naturally infected samples of similar varieties, inoculated samples vs naturally infected samples of different varieties and inoculated samples vs naturally infected samples of unknown variety (market samples) as described in the **Supplementary materials**.

3.0 RESULTS

3.1 Aflatoxin contamination levels for maize artificially inoculated with *A. flavus*

Aflatoxin contamination levels in parts per billion (ppb) were significantly higher (p = 0.0002, n = 90) in Duma 43 samples inoculated with the aflatoxigenic *A. flavus* isolate (158 ± 137 ppb) when compared to the controls (0.6 ± 0.3 ppb) and samples inoculated with the non-aflatoxigenic isolates (0.4 ± 0.2ppb). Similarly for Pioneer, aflatoxin contamination levels were significantly higher (p <0.001, n = 90) for samples inoculated with aflatoxigenic *A. flavus* isolate (885.75 ± 158 ppb) when compared to the controls (0.1± 0.05 ppb) and samples

inoculated with the non-aflatoxigenic isolates (0.7 ± 0.3ppb). The control and non-aflatoxigenic samples contained aflatoxins levels that were below the maximum set regulatory limit of 5 ppb [25] for maize and therefore these samples were considered as true negative controls for the purpose of this study.

3.2 Aflatoxin contamination levels for maize naturally infected with *A. flavus*

Maize samples potentially infected with *A. flavus* under field conditions were purchased from farms in aflatoxin prone areas in Kenya and included the main varieties grown in those respective areas. Three varieties were obtained. Two of these; Duma 43 and Pioneer, were the same as those experimentally inoculated with *A. flavus.* The third variety was DH04. Market samples were also purchased, but their varieties were not known. Aflatoxin contamination levels were higher for samples purchased from the market when compared to individual varieties grown and purchased from farms. Contamination levels ranged from 49.8 ppb for Duma 43 to 12,680 ppb for market samples **(Table 2)**. For the purpose of this study samples with aflatoxin levels between 0 to 5.9 ppb were considered as uncontaminated, whereas samples with contamination levels equal to or greater than 6 pbb were classified as contaminated.

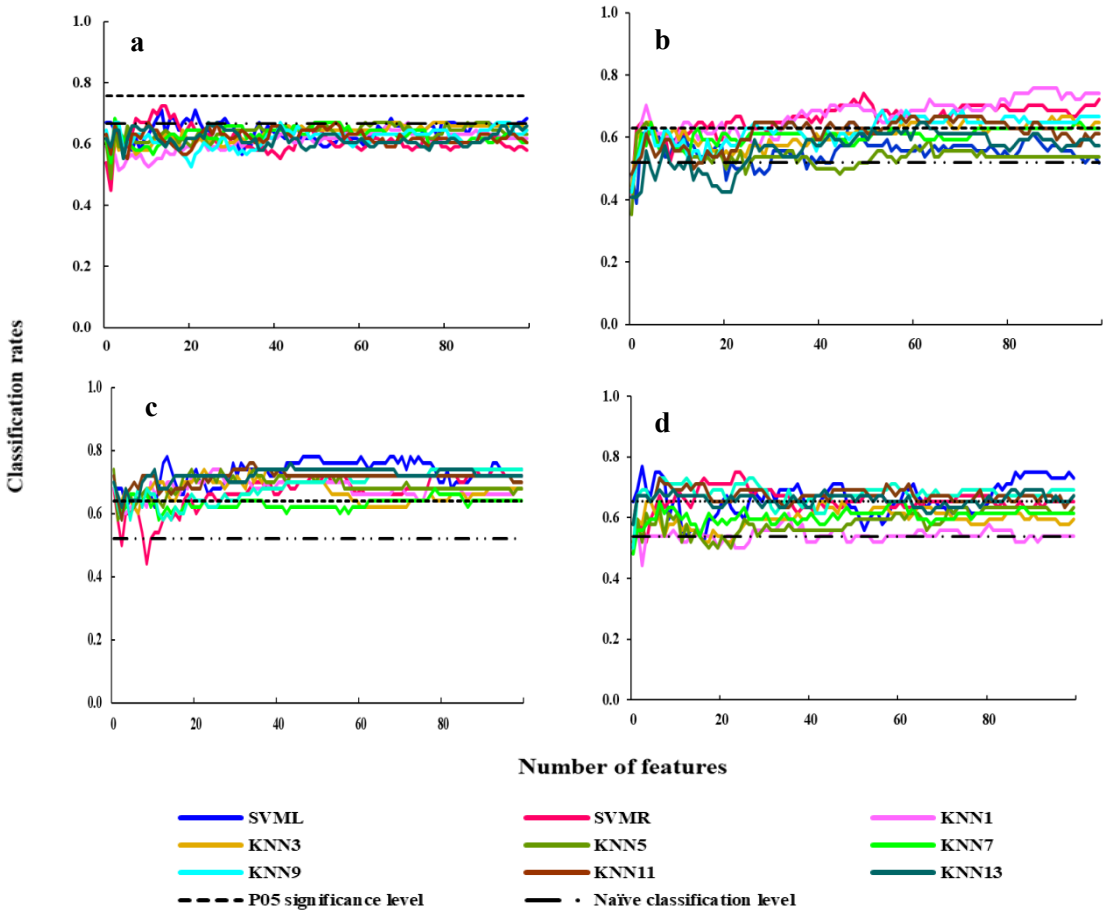
Table 2. Aflatoxin contamination levels in parts per billion (ppb) of Kenyan maize varieties and market samples naturally infected with *A. flavus* under field conditions.

Variety	**Number of samples with different aflatoxin levels**			**Average aflatoxin levels in ppb**
	Below regulatory limit of 5ppb	**Above regulatory limit of 5 ppb**	**Total samples**	
DHO4	41	11	52	49.8
Pioneer	34	31	65	100
Duma 43	22	13	35	269
Market samples	23	20	43	12,680
Total	**120**	**75**	**195**	

*Market samples – Mixture of unknown varieties

3.3 Classification performance for DiagNose analysis Kenyan maize varieties artificially inoculated with *A. flavus*

The potential for DiagNose to detect aflatoxin contamination in the maize artificially inoculated with *A. flavus* was evaluated by the ability of the sensors to discriminate between the different sample classes, namely control, non-aflatoxigenic *A. flavus* infected maize and aflatoxigenic *A. flavus* infected maize. Results for MI between the classes are presented as a heat map showing the sensor responses to the different samples (**Supplementary materials)**. The average correct classification rate was plotted against the number of features for each class comparison to determine the classification performance. The number of classifiers (of the nine total) that achieved classification rates equal to or above the P05 significance level (95 %) and how the classification rates were sustained across the different number of features determined the robustness of the classification performance. Classification rates sustained across the different numbers of features indicated a robust classification performance the nine classifiers tested**.** For Duma 43, three of the four class comparisons (except control vs all *A. flavus* infected maize) had classifiers achieving classification accuracies equal to or above the P05 significance level **(Figure 1)**. The classification rates for these three comparisons were also sustained across the different numbers of features indicating a robust classification performance.

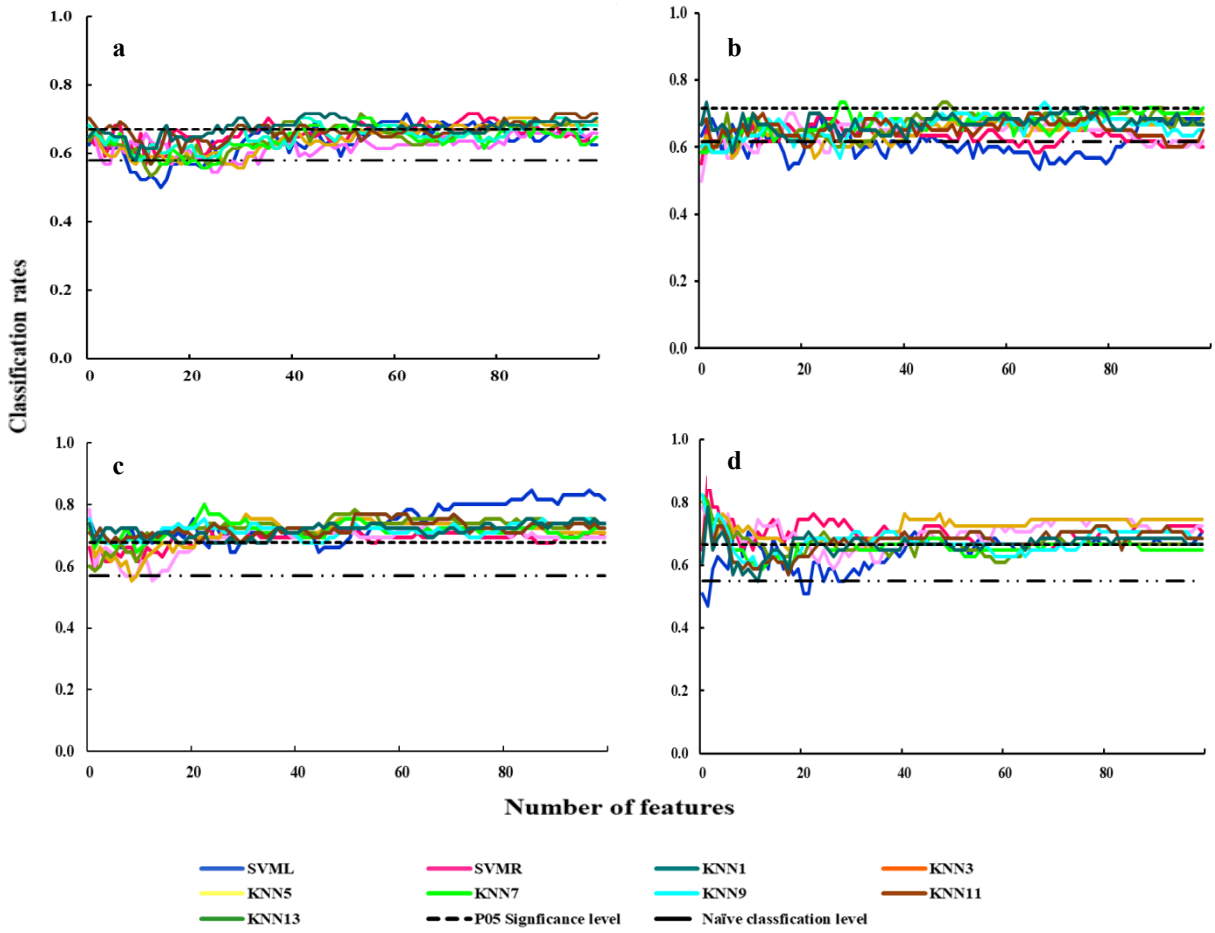


SVML- Support vector machines- linear function; SVMR- Support vector machines- radial function; KNN - *k*-nearest neighbour with different values of *k* (1, 3, 5, 7, 9, 11, 13); Classification rate above or equal to the P05 significance level is significant; Naïve classification level represents random classification (by chance).

Figure 1: Cross-validated classification accuracy versus number of features selected by mutual information for Kenyan maize variety Duma 43; **(a)** control vs all *A. flavus* infected maize flour, **(b)** control vs aflatoxigenic *A. flavus* infected maize flour, **(c)** control vs non-aflatoxigenic *A. flavus* infected maize flour and **(d)** non-aflatoxigenic vs aflatoxigenic *A. flavus* infected maize flour. ***Colour printing required**

The DiagNose achieved classification rates equal to or above the P05 significance level for all the class comparisons with some of the classifiers for Pioneer. A robust classification performance was achieved for the control vs non-aflatoxigenic *A. flavus* infected maize and non-aflatoxigenic vs aflatoxigenic *A. flavus* infected maize. The classification rates were however not sustained across the different numbers of features indicating a non-robust

classification performance for the control vs all *A. flavus* infected maize and control vs aflatoxigenic *A. flavus* infected maize **(Figure 2).**



SVML- Support vector machines- linear function; SVMR- Support vector machines- radial function; KNN - *k*-nearest neighbour with different values of *k* (1, 3, 5, 7, 9, 11, 13); Classification rate above or equal to the P05 significance level is significant; Naïve classification level represents random classification (by chance).

Figure 2: Cross-validated classification accuracy versus number of features selected by mutual information for Kenyan maize variety Pioneer; **(a)** control vs all *A. flavus* infected maize flour, **(b)** control vs aflatoxigenic *A. flavus* infected maize flour, **(c)** control vs non-aflatoxigenic *A. flavus* infected maize flour and **(d)** non-aflatoxigenic vs aflatoxigenic *A. flavus* infected maize flour. ***Colour printing required**

3.5 Classification accuracy for DiagNose analysis Kenyan maize varieties artificially inoculated with *A. flavus*

The results demonstrate the potential for DiagNose to discriminate between controls and samples artificially inoculated with *A. flavus* for the two varieties. Cross-validated classification accuracies achieved ranged from 72 % to 88 % for the two varieties across the

different class comparisons **(Table 3)**. Classification accuracies across the different class comparisons for both varieties were significant for some classifiers ($P \geq 05$) in all except control vs all *A. flavus* infected maize for Duma 43. The best classifiers for all class comparisons for the two varieties were higher than the naive classification level which represents random classification **(Table 3)**. Classification accuracy for the control vs all *A. flavus* infected maize and control vs aflatoxigenic *A. flavus* maize, which represent field conditions, ranged from 72 % to 76 % for Duma 43 and from 72 % to 73 % for Pioneer. SVM was superior to KNN in classification of the different sample types. SVM recorded best correct classification rates for three out of the four pairwise comparisons for the two varieties **(Table 3)**. A higher proportion of false positives relative to false negatives was recorded across the different class comparisons for the two varieties, except for classification of control vs all *A. flavus* infected maize **(Table 3)**. In practice this implies that the DiagNose would classify more of the uncontaminated samples as contaminated than it would classify contaminated samples as non-contaminated which is less detrimental to the consumers.

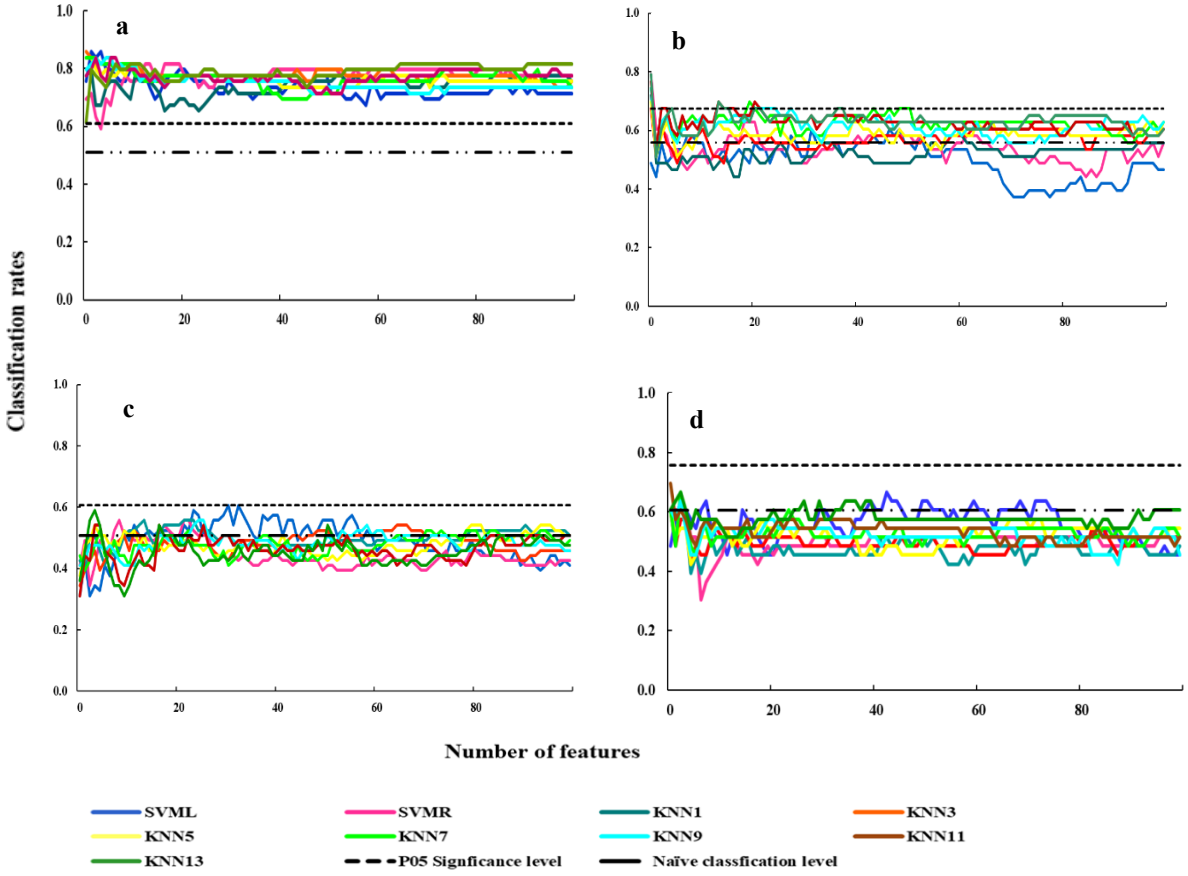
Table 3: Cross-validated best classification accuracy for DiagNose analysis of maize flour for Kenyan maize varieties Duma 43 and Pioneer inoculated with 2 % Tween 20 as a control and non-aflatoxigenic and aflatoxigenic isolates of *A. flavus* across different class comparisons.

Variety	Class comparisons	*No. of samples	Classification (%)	P05 significance level (%)	Naïve classification (%)	No. classifiers ≥ P05 significance	Best classifier	Misclassification type	
								False +ve	False –ve
Duma 43	Control vs all *A. flavus*	76	72	76	67	0/9	SVM	10	11
	Control vs aflatoxigenic	54	76	63	52	9/9	KNN	7	6
	Control vs non-afla	50	78	64	52	9/9	SVM	6	5
	Non-afla vs aflatoxigenic	52	77	65	54	7/9	SVM	10	2
Pioneer	Control vs all *A. flavus*	88	72	67	58	9/9	SVM	11	14
	Control vs aflatoxigenic	60	73	72	62	3/9	KNN	9	7
	Control vs non-afla	60	85	68	57	9/9	SVM	6	3
	Non-afla vs aflatoxigenic	51	88	67	55	9/9	SVM	3	3

* Due to limited amount of maize flour obtained after milling, the number of samples used for analysis were lower than the number of inoculated cobs. Class comparison- all *A. flavus*- All *A. flavus* infected maize flour, aflatoxigenic- aflatoxigenic *A. flavus* infected maize flour, non-aflatoxigenic- non-aflatoxigenic *A. flavus* infected maize flour; No. of samples – Number of samples used for analysis; Non-afla- non-aflatoxigenic; Classification (%)-Highest classification rate recorded; P05 significance level (%) - significance level at 95 % confidence level; Naïve classification- classification expected by chance; No. ≥ P05 significance- Number of classifiers out of the total nine that achieved classification rates equal to or greater than the P05 significance level; Best classifier- Classifier that achieved the highest classification rate, SVM- Support vector machines; KNN - *k*-nearest neighbour; False +ve - Number of false positive samples (control samples classified as either *A. flavus* infected, aflatoxigenic or non-Aflatoxigenic); No.- Number; False –ve- Number of false negative samples (*A. flavus* infected, aflatoxigenic and non-aflatoxigenic samples classified as control).

3.6 Classification performance for DiagNose analysis Kenyan maize varieties with *A.* naturally infected with *A. flavus*

The capability of DiagNose to detect aflatoxin in naturally contaminated Kenyan maize varieties was evaluated by the ability of the sensors to correctly differentiate uncontaminated (< 6 ppb aflatoxin) and contaminated (≥ 6 ppb aflatoxin) samples. Results for MI between the classes are presented as a heat map showing the sensor responses to the different samples (**Supplementary materials)**. This capability was not consistent for the three varieties and the market samples. All classifiers tested achieved statistically significant classification rates for DH04 which were sustained across the different number of features **(Figure 3)**. For market samples, although eight out of the nine classifiers tested achieved classification rates above or equal to the P05 significance level, the rates were achieved with only one feature and were not sustained across the different number of features indicating a weak/non-robust classification performance. DiagNose was least effective in discriminating contaminated from uncontamianted samples for Duma 43 and Pioneer. For Duma 43 the classification rates for all classifiers were below the P05 significance level, while for Pioneer only one classifier out of the nine tested achieved classificaton rate equal to the P05 significance level which was not suitained across the different number of features.



SVML- Support vector machines- linear function; SVMR- Support vector machines- radial function; KNN - *k*-nearest neighbour with different values of *k* (1, 3, 5, 7, 9, 11, 13); Classification rate above or equal to the P05 significance level is significant; Naïve classification level represents classification by random chance.

Figure 3: Cross-validated classification accuracy versus number of features selected by MI for Kenyan maize varieties **(a)** DH04, **(b)** Market samples (mixture of unknown varieties), **(c)** Pioneer and **d)** Duma 43 samples naturally infected with *A. flavus* under field conditions.
***Colour printing required**

3.7 Classification accuracy for DiagNose analysis Kenyan maize varieties artificially inoculated with *A. flavus*

The capability of DiagNose under some circumstances to detect aflatoxin contamination in maize samples naturally infected with *A. flavus* was demonstrated. Highest classification accuracies for both known varieties and market samples ranged from 61 % to 86 % **(Table 4)**. Classification accuracy was highest at 86 % and more robust for DH04 compared to the other varieties and the market samples. A statistically significant classification accuracy of 79 % was also achieved for market samples, which are expected to be the most difficult to classify, considering they are a mixture of different and unknown varieties. The samples represent field conditions in the market where maize flour sold to consumers in retail outlets comprises

a mixture of different unknown varieties. For Duma 43 the highest classification rate was below the P05 significance level and only 70 % of the samples were accurately classified while for Pioneer only 61 % of the samples were accurately classified. SVM was more effective than KNN in classification of maize samples naturally infected with *A. flavus*. SVM achieved highest classification rates for two out of three varieties evaluated and the market samples while KNN gave a non-significant best correct classification rate for Duma 43. The number of false negatives was higher than false positives across the different varieties and market samples indicating that the DiagNose has a higher chance to misclassify samples that are naturally infected with *A. flavus* as non-contaminated when they are actually contaminated.

Table 4: Cross-validated best classification accuracy for DiagNose analysis of flour from Kenyan Maize varieties Duma 43, Pioneer, DH04 and Market samples naturally infected with *A. flavus* under field conditions.

Variety	*No. of samples	Classification (%)	P05 significance level (%)	Naïve classification level (%)	No. classifiers ≥ P05 significance level (%)	Best Classifier	Misclassification type	
							False +ve	False –ve
Duma 43	33	70	76	60	0/9	KNN	4	6
Pioneer	64	61	61	51	1/9	SVM	12	13
DH04	51	86	61	51	9/9	SVM	3	4
Market samples	43	79	67	56	8/9	SVM	3	6

No. of samples – Number of samples used for analysis; Classification (%)-Highest classification rate recorded; P05 significance level (%) - significance level at 95% confidence level; Naïve classification- classification expected by chance depending on the number of samples in each class; Number classifiers ≥ P05 significance- Number of classifiers out of the total nine that achieved classification rates equal to or greater than the P05 significance level; Classification-Classifier that achieved the highest classification rate; SVM Support Vector Machines; KNN - *k*-nearest neighbour; No.- Number; False +ve - Number of false positive samples (control samples classified as contaminated); False –ve- Number of false negative samples (contaminated sample classified as control).

3.5 External validation of DiagNose data

Cross-validated best correct classification rates achieved with external validation were compared with classification rates achieved previously with internal validation for samples naturally infected with *A. flavus* in a number of training and validation sets combinations **(Table 5)**. In all except one case (Pioneer inoculated vs Pioneer naturally infected), the external validation was weaker than the leave-one out cross-validation (internal validation). Classification rates achieved during internal validation showed the capability of DiagNose to discriminate between uncontaminated and contaminated maize samples naturally infected with *A. flavus* with accuracies ranging from 61 % to 86 %. External validation of the data yielded classification accuracies that ranged from 62 % to 78 % across the different training and validation set combinations. Classification accuracies were statistically significant for four out of seven training/validation set combinations that included; inoculated samples vs naturally infected samples of similar varieties for Pioneer, inoculated samples (Duma 43 and Pioneer) vs naturally infected samples of different variety (DH04) naturally infected with *A. flavus,* and Duma 43 samples artificially inoculated with *A. flavus* used for external validation of data from market samples naturally infected with *A. flavus*. Classification accuracies were however, non-significant when data from all artificially infected samples were used for external validation for all naturally infected samples, when Duma 43 artificially inoculated data were used for external validation of Duma 43 naturally infected with *A. flavus* and when Pioneer data for artificially inoculated samples were used for external validation of market samples naturally infected with *A. flavus*. Though the classification rates were below the significant level, they were all above the naïve classification level.

Table 5: Cross-validated accuracies for external validation of potential for DiagNose to detect aflatoxin contamination in maize flour using data from maize samples artificially infected with *A. flavus* as a training set and data from maize samples naturally infected with *A. flavus* as validation set.

Description	Training set	Validation/test set	Internal validation (Validation set)	External validation (Training: validation set)		
			Best correct classification (%)	Best correct classification (%)	P05 significance level (%)	Naive classification level (%)
All samples	All AI	All NI	68	62	64	58
Similar Varieties	Duma-AI	Duma-NI	70	67	76	60
	Pioneer-AI	Pioneer-NI	61	64	61	51
Different varieties	Duma-AI	DH04-NI	86	76	61	51
	Pioneer-AI	DH04-NI	86	78	61	51
***Market samples**	Duma-AI	Market samples-NI	79	74	67	56
	Pioneer-AI	Market samples-NI	79	58	67	56

AI – Samples artificially inoculated with *A. flavus*; NI- Samples naturally infected with *A. flavus* under field conditions.

4.0 Discussion and Conclusion

The capability of a field portable electronic nose (DiagNose) combined with mutual information as a feature selection method for detecting aflatoxin contamination in maize artificially inoculated and naturally infected with *A. flavus* was evaluated. Successful discrimination of controls from maize artificially inoculated with aflatoxigenic and non-aflatoxigenic *A. flavus* under laboratory conditions was demonstrated with two Kenyan maize varieties. The DiagNose was also able to discriminate between aflatoxin contaminated and uncontaminated Kenyan maize varieties that were naturally infected with *A. flavus* under field conditions in some circumstances. Additionally, external validation of the DiagNose yielded classification accuracies for aflatoxin contamination that were relatively consistent with accuracies obtained from internal validation.

Due to the complex and non-linear nature of electronic nose data, KNN and SVM are the most widely used classifiers [26]. In this study, SVM achieved the highest classification accuracies in most instances. The superior performance of SVM compared to KNN could be attributed to its inherent ability to deal with non-linear complex separation problems through application of the kernel function that enables transformation of the data from a low dimension space to a higher dimensional space where the classes can be separated [27]. Similar results were reported by Chen, Zhao [28] while evaluating the potential of an electronic nose for discrimination of green tea quality. The study compared SVM, KNN and an artificial neural network for discrimination of four grades of green tea. Optimum discrimination was achieved with SVM in comparison to KNN and the artificial neural network.

External validation is conducted to evaluate the predictive accuracy of the classification model with datasets that were not used to train the model and its potential for generalizability to future settings [29]. In the context of this study, a laboratory validation was conducted to ascertain the possibility of training an electronic nose with laboratory generated samples and achieving correct classification with field samples. Though the external validation was generally weaker than leave-one out cross-validation, statistically significant classification accuracies were achieved with external validation in some circumstances. The results indicate the possibility that DiagNose could be trained with maize samples artificially contaminated with aflatoxins in the laboratory and then be used to classify field samples that are naturally contaminated with aflatoxins for varieties similar to those used for training, varieties that are different to those used for training and mixtures of unknown varieties/market samples. However, there is a need to validate the findings with a wider range of maize varieties and

training-validation combinations than used in this study. To the best of our knowledge, this is the first study reporting external validation of the potential for an electronic nose to detect aflatoxin contamination in maize. It is however, consistent with previous studies on external validation of the potential application of electronic nose as a diagnostic technique for detection of contamination on other food matrices [30].

The results indicate the potential for DiagNose, supported by mutual information, as a field portable instrument for detection of aflatoxin contamination of maize. Classification accuracies ranged from 72 % to 88 % for maize samples artificially inoculated with *A. flavus* and 61 % to 86 % for maize samples naturally infected with *A. flavus* under field conditions. The results are consistent with findings from other studies evaluating the potential application of electronic nose to detect aflatoxin contamination of maize. Ottoboni, Pinotti [12] achieved an overall 65 % cross-validated classification accuracy for detection of aflatoxin and fumonisin contamination of maize samples that were either below the regulatory limit, singly contaminated or co-contaminated using an electronic nose equipped with an array of ten metal oxide semiconductor sensors and a nine variate discriminant function analysis model. Leggieri, Marco [13] using an electronic nose with an array of ten metal oxide semiconductor sensors and an artificial neural network reported discrimination of maize grains contaminated above or below the legal limit for aflatoxin B1 with an accuracy of 78 %.

Classification accuracies varied amongst the different varieties. This could be an indication that potential for DiagNose to discriminate between aflatoxin contaminated and uncontaminated field samples could be variety dependent. A possible explanation is the inherent genetic differences between the varieties which could have an influence on the type and quantity of VOCs produced and hence on the capability of DiagNose to detect contamination. Variations in VOC profiles among maize and wheat varieties infected with the same species of mycotoxigenic fungi has been previously reported [18, 19, 31]. The maize samples were collected from farmers in different agro-ecological zones in Kenya and were presumably produced under different ecological, agronomic and climatic conditions, which could also account for the variability in VOCs produced as reported by Gouinguene´ and Turlings [32]. Additionally, varieties could also have been infected with different strains of *A. flavus* which have been documented to vary across the different agro ecological zones in Kenya [20, 33-35].

In ideal circumstances, the goal is to have a diagnostic technique that can perfectly discriminate between aflatoxin contaminated and uncontaminated samples. However, 100 % accuracy is rare for any diagnostic, values close to 100 % are considered acceptable [36]. The classification accuracies achieved in this study, though statistically significant in some cases and better than chance, are too low to justify deployment of DiagNose as a field diagnostic for detection of aflatoxin contamination, which would require correct classification rates of greater than 90 %. A lower level of classification success may be acceptable for a screening test but only if the false negative rate is low. Unlike the maize samples artificially inoculated with *A. flavus*, a higher proportion of false negatives compared to false positives was recorded for the maize samples naturally infected with *A. flavus* under field conditions. This is not ideal for a screening technique. A bias towards a false positive result is preferable because few genuinely contaminated samples could be released for consumption. A high proportion of false negatives would be less safe because more contaminated samples would be released for consumption.

The results do, however, provide important initial insights and a proof of concept of the potential application of electronic nose technology in general, and the DiagNose in particular, for detecting aflatoxin contamination in maize. Our results indicate that the DiagNose can correctly discriminate between aflatoxin contaminated and uncontaminated samples in 70 out of 100 samples on average. This, coupled with the field portability of the instrument and the possible detection of contamination without sample preparation, would allow for rapid, efficient and cost-effective initial screening on a large number of samples on a 'Yes' or 'No' criterion basis. The objective would be to reduce the number of samples required to undergo more expensive and time-consuming quantitative analyses. This could translate into reduced cost and time of analysis, which would facilitate timely removal of contaminated lots from the food and/or feed chains.

There is need, however, for further research to improve the classification accuracy and performance of electronic noses before they can be adopted for practical use. A number of challenges related to the performance of the DiagNose need to be addressed. These include selectivity and redundancies of sensors, where sensors with poor selectivity adversely affect the discriminating power of the array. The future applicability of electronic noses for detection of aflatoxins in maize is therefore dependent on selection of the most responsive sensors for detection of aflatoxin contamination across a wide range of samples including intact kernels. The selected sensors could be utilized for formulation of an optimized instrument potentially a probe with fewer sensors that are more selective hence more

effective and accurate in detecting aflatoxin contamination. The optimized instrument when available would be an innovative, cost-effective and easy to use technique for detection of aflatoxin contamination along the different points of the maize supply chain. It would not require highly skilled operators, hence well suited for rural areas where there is very little or non-existent laboratory infrastructure.

Acknowledgement

We thank the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Bioscience eastern and central Africa (BecA-ILRI Hub) staff for their valuable contributions to this project. We also thank the National Irrigation Authority (NIA Kenya) for providing the maize varieties, the Ministry of Agriculture Kenya- Kaiti Sub County for assistance with field sample collection and the University of Nairobi School Of Biological Sciences for providing the *Aspergillus flavus* isolates.

Funding

This work was supported by the Australia Awards-Africa, administered by the Department of Foreign Affairs and Trade and CSIRO's Health and Biosecurity flagship.

Author's Contribution

Catherine .W. Machungo; Conceptualization, Methodology, Investigation, Validation, Investigation, Data Curation, Writing- Original draft and Visualization. **Amalia Z. Berna;** Conceptualization, Methodology, Validity, Data Curation, Writing- Review and Editing and Supervision. **Dennis McNevin;** Conceptualization, Methodology, Validity, Writing- Review and editing and Supervision. **Rosalind Wang;** Software, Formal analysis and Data curation. **Jagger Harvey;** Conceptualization, Methodology and Funds Acquisition. **Stephen Trowell;** Conceptualization, Methodology, Writing- Review and Editing and Supervision and Funds Acquisition.

Declarations of interest:

The authors have declared no competing interest.

References

[1] Birgen, J.K., R.C. Cheruiyot, and T.E. Akwa, Mycotoxin contamination of stored maize in Kenya and the associated fungi. *Journal of Plant Pathology Research, 2020. 1(1)*: p. 7-13. doi: 10.36959/394/620

[2] Agriopoulou, S., E. Stamatelopoulou, and T. Varzakas, Advances in occurrence, importance and mycotoxin control strategies: Prevention and detoxification in foods. *Foods 2020.* ***9****(137)*: p. 2-48. doi: https://doi.org/10.3390/foods9020137

[3] Omara, T., et al., The scourge of aflatoxins in Kenya: A 60-year review (1960 to 2020). *Journal of Food Quality, 2021. 21*: p. 1-31. https://doi.org/10.1155/2021/8899839

[4] Wu, F. and P. Khlangwiset, Evaluating the technical feasibility of aflatoxin risk reduction strategies in Africa. *Food Additives Contamination Part A Chemical Analytical Control Exposed Risk Assessment, 2010.* ***27****(5)*: p. 658–676. doi:https://doi.org/10.1080/19440041003639582

[5] Cheli, F., et al., Feed analysis and animal nutrition: Electronic nose as a diagnostic tool. *Chemical Engineering Transactions, 2018. 68(2018)*: p. 1-6. doi:https://doi.org/10.3303/CET1868038

[6] Falasconi, M., et al., Review Article. Electronic nose for microbiological quality control of food products. *International Journal of Electrochemistry, 2012*: p. 1-12. doi:https://doi.org/10.1155/2012/715763

[7] Olsson, J., et al., Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. *International Journal of Food Microbiology, 2002.* ***72****(3)*: p. 203-214. doi:https://doi.org/10.1016/S0168-1605(01)00685-7

[8] Paolesse, R., et al., Detection of fungal contamination of cereal grain samples by an electronic nose. *Sensors and Actuators B: Chemical, 2006. 119(2)*: p. 425-430. doi:https://doi.org/10.1016/j.snb.2005.12.047

[9] Campagnoli, A., et al., Use of the electronic nose as a screening tool for the recognition of durum wheat naturally contaminated by deoxynivalenol: A preliminary approach. *Sensors, 2011. 11(5)*: p. 4899-4916. doi:https://doi.org/10.3390/s110504899

[10] Gobbi, E., et al., Electronic nose predicts high and low fumonisin contamination in maize cultures. *Food Research International, 2011. 44(4)*: p. 992-999. doi: https://doi.org/10.1016/j.foodres.2011.02.041

[11] Lippolis, V., et al., Screening of deoxynivalenol contamination in durum wheat by MOS-based electronic nose and identification of the relevant pattern of volatile compounds. *Food Control, 2014.* ***37****(Supplement C)*: p. 263-271. doi:https://doi.org/10.1016/j.foodcont.2013.09.048

[12] Ottoboni, M., et al., Combining E-nose and lateral flowimmunoassays (LFIAs) for rapid occurrence/co-occurrence of aflatoxin and fumonisin detection in maize. *Toxins, 2018.* ***10****(416)*: p. 1-11. doi: https://doi.org/10.3390/toxins10100416

[13] Leggieri, M.C., et al., An electronic nose supported by an artificial neural network for the rapid detection of aflatoxin B1 and fumonisins in maize. *Food Control 2020. 107722*: p. 1-8. doi:https://doi.org/10.1016/j.foodcont.2020.107722.

[14] Cheli, F., et al., Electronic nose for determination of aflatoxins in maize. *Biotechnology Agronomy Society and Enviroment., 2009. 13(S)*: p. 39-43. doi:https://popups.uliege.be/1780-4507/index.php?id=3522

[15] Sun, D., et al., Effects of growth parameters on the analysis of *Aspergillus flavus* volatile metabolites. *Separations 2016. 3(13)*: p. 1-20. doi: https://doi.org/10.3390/separations3020013

[16] De Lucca, J.A., et al., Volatile profiles and aflatoxin production by toxigenic and non-toxigenic isolates of *Aspergillus flavus* grown on sterile and non-sterile cracked corn. *Annals of Agricultural and Environmental Medicine, 2012. 19(1)*: p. 91-98.

[17] Jurjevic, Z., et al., Volatile metabolites associated with one aflatoxigenic and non aflatoxigenic *Aspergillus flavus* strain grown on two different substrates. *Phytopathology Mediterranean, 2008. 47*: p. 266-271. doi: https://doi.org/10.14601/Phytopathol_Mediterr-2731

[18] Busko, M., et al., Comparison of volatiles profile and contents of trichothecenes group B,ergosterol and ATP of bread wheat,durum wheat,and triticale grain naturally contaminated by mycobiota. *Frontiers in Plant Science 2016. 7(1243)*: p. 1-16. doi: https://doi.org/10.3389/fpls.2016.01243

[19] Sherif, M., et al., Volatiles emitted from maize ears simultaneously infected with two Fusarium species mirror the most competitive fungal pathogen. *Frontiers in Plant Science 2016 7(1460)*: p. 1-11. doi: https://doi.org/10.3389/fpls.2016.01460

[20] Okoth, S., et al., Toxigenic potential of *Aspergillus* species occurring on maize kernels from two Agro-ecological zones in Kenya. *Toxins, 2012. **4***: p. 991-1007. doi: https://doi.org/10.3390/toxins4110991

[21] Machungo, C., et al., Comparison of the performance of metal oxide and conducting polymer electronic noses for detection of aflatoxin using artificially contaminated maize. *Sensors and Actuators B: Chemical, 2022. 360*: p. 131681. doi:https://doi.org/10.1016/j.snb.2022.131681

[22] Yarda, E.E., et al., Human aflatoxin exposure in Kenya, 2007: A cross-sectional study. *Food Additives & Contaminants: Part A, 2013. 30(7)*: p. 1322–1331. doi:https://doi.org/10.1080/19440049.2013.789558

[23] Maina, A.W., et al., Post harvest practices for farmers in Kaiti district Kenya, and the impact of hermatic storage on population of *Asperigillus* spp and aflatoxin contamination. *Journal of Food Research, 2016. **5**(6)*: p. 53-66. doi: 10.5539/jfr.v5n6p53

[24] Probst, C., et al., Identification of atoxigenic *Aspergillus flavus* isolates to reduce aflatoxin contamination of maize in Kenya. *Plant Disease, 2011. 95*: p. 212-218. doi: https://doi.org/10.1094/PDIS-06-10-0438

[25] Sirma, A.J., et al., The impacts of aflatoxin standards on health and nutrition in sub-Saharan Africa: The case of Kenya. *Global Food Security 2018. **18***: p. 57-61. doi: https://doi.org/10.1016/j.gfs.2018.08.001

[26] Berrueta, L.A., R.M. Alonso-Salces, and K. Héberger, Supervised pattern recognition in food analysis. *Journal of Chromatography A, 2007. 1158(1)*: p. 196-214. doi:https://doi.org/10.1016/j.chroma.2007.05.024

[27] Tan, J. and J. Xu, Applications of electronic nose (e-nose) and electronic tongue (e-tongue) in food quality-related properties determination: A review. *Artificial Intelligence in Agriculture, 2020. 4*: p. 104-115. doi:https://doi.org/10.1016/j.aiia.2020.06.003

[28] Chen, Q., et al., Discrimination of green tea quality using the electronic nose technique and the human panel test, comparison of linear and nonlinear classification tools. *Sensors and Actuators B: Chemical, 2011. 159(1)*: p. 294-300. doi:https://doi.org/10.1016/j.snb.2011.07.009

[29] Consonnia, V., D. Ballabioa, and R. Todeschini, Evaluation of model predictive ability by external validation techniques. *Journal of Chemometrics 2010. 24*: p. 194-201. doi:https://doi.org/10.1002/cem.1290

[30] Lippolis, V., et al., Rapid prediction of ochratoxin A-producing strains of Penicillium on dry-cured meat by MOS-based electronic nose. *International Journal of Food Microbiology, 2016. 218*: p. 71-77. doi:https://doi.org/10.1016/j.foodcont.2013.09.048

[31] Fauguel, C.M., et al., Volatile compounds released by maize grains and silks in response to infection by *Fusarium verticillioides* and its association with pathogen resistance. *Plant Pathology 2017. 66*: p. 1128- 1138. doi:https://doi.org/10.1111/ppa.12663

[32] Gouinguene´, S.P. and T.C.J. Turlings, The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology 2002. 129*: p. 1296-1307. doi: https://doi.org/10.1104/pp.001941

[33] Odhiambo, B.O., H. Murage, and I.N. Wagara, Isolation and characterisation of aflatoxigenic *Aspergillus* species from maize and soil samples from selected counties of Kenya. *African Journal of Microbiology Research, 2013. **7(34)***: p. 4379-4388. doi:https://doi.org/10.5897/AJMR2013.5846

[34] Okoth, S., et al., Genetic and toxigenic variability within *Aspergillus flavus* population isolated from maize in two diverse environments in Kenya. *Frontiers in Microbiology, 2018. **9(57)***: p. 1-14. doi:https://doi.org/10.3389/fmicb.2018.00057

[35] Salano, N.E., et al., Diversity of putatively toxigenic *Aspergillus* species in maize and soil samples in an aflatoxicosis hotspot in Eastern Kenya. *African Journal of Microbiology Research 2016. **10(6)***: p. 172-184. doi: https://doi.org/10.5897/AJMR2015.7645

[36] van Stralen, K.J., et al., Diagnostic methods : Sensitivity, specificity, and other measures of accuracy. *Kidney International, 2009. **75(12)***: p. 1257-1263. doi:https://doi.org/10.1038/ki.2009.92