Electronic nose for early detection of basal stem rot caused by \textit{Ganoderma} in oil palm

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Abstract. The successful control of basal rot disease (BSR) determined by early detection of infection because when the symptoms already appear, generally plants are difficult to save. The earlier the \textit{Ganoderma} infection is known, the easier the control will be and the losses can be minimized. Therefore, early detection of \textit{Ganoderma} infection is very necessary, which in this study was carried out by detecting volatile compounds using electronic nose (E-nose). E-nose detection has been carried out to analyze the compounds formed in pure \textit{Ganoderma} culture. Detection of plants in the field carried out at 4 levels of infection, i.e. healthy, early, moderate and severe infection. The results concluded that \textit{Ganoderma} mycelium when compared with other fungi (\textit{Trichoderma}, \textit{Aspergillus} and \textit{Omphalina}) showed significant differences when analyzed using an unsupervised PCA chemometric system. The E-nose data processed using machine learning Support Vector Machine (SVM) was able to distinguish the aroma between \textit{Ganoderma boninense} CSB, \textit{G. boninense} ‘Rejosari’, and \textit{G. lucidum} with an accuracy rate of 99.64%. E nose was able to differentiate with high accuracy (90.95%) of each infection level even though there was still a slice between in root sample.

1. Introduction
Basal stem rot disease (BSR) caused by \textit{Ganoderma sp.} is the most important disease in oil palm plantations. The disease caused devastating damage due to direct loss of the stand, reduced yield of diseased palms and the resultant requirement for earlier replanting. \textit{Ganoderma boninense} [1] not only causes BSR, but also upper stem rot (USR). When young oil palms show symptoms of the disease, the plants usually die within 1 or 2 yr, while mature trees can survive for only another 3 or so yr. The successful control of BSR determined by early detection of infection because when the symptoms appear, generally plants are difficult to be saved since pathogenic infections have spread to all parts of the plants. The earlier the \textit{Ganoderma} infection is known, the easier the control will be and the losses can be minimized [2, 3]. Therefore, early detection of \textit{Ganoderma} infection is very necessary, which in this study was carried out by detecting volatile compounds using electronic nose (E-nose) [4]. The E-nose technique has been used to detect fungal infections in blueberries, tomatoes and wheat, and identification techniques have been obtained for \textit{Penicillium}, \textit{Aspergillus} and \textit{Fusarium} infections [5,
The objective of this technology is to mimic the mammalian sense of smell by producing a composite response that is unique to each odorant. The odors detect by an array of sensors and transduce into electrical signals that can be successively analysed by using pattern recognition strategies [8]. Previous research showed that based on PCA plots from the samplings experiment, the samples were qualitatively clustered in 2 groups, odour from basidiocarp of *G. boninense* and ambient air [9]. Previously, those research group has successfully completed an initial investigation to differentiate BSR-infected and healthy oil palm trees [10]. In this research, E-nose designed by research group from Gadjah Mada University had been carried out to analyze the compounds formed in pure *Ganoderma* culture, and oil palm plants-infected *Ganoderma* in the field. Detection of plants in the field were carried out at 4 levels of infection, namely healthy, mild, moderate and severe infection.

2. Methods

2.1. Electronic nose apparatus and analysis

A laboratory-made E-nose with 8 MOS gas sensors (Taguchi gas sensor/TGS series) was used in-situ (at the production line of the tea factory), for real-time analysis of tea samples. The environment temperature and relative humidity during the measurements were 21 ± 2°C and 85 ± 10%, respectively. TGS 2620, TGS 2612, TGS 832, TGS 822, TGS 2603, TGS 2600, TGS 813, and TGS 826 were the global selectivity of MOS gas sensors comprised in the device. Besides the gas sensor array, the built E-nose device comprised a sampling system, a data acquisition unit (DAQ), and a signal processing framework, as shown in Fig. 1. The sampling system comprises 2 electronic valves (three-way system) for air flow control.

![Figure 1](image_url)

**Figure 1.** (a) The photograph of E-nose device used in this study (A, computer with chemometric tools; B, main part of the E-nose devices; and C, chamber of sample) and (b) Schematic diagram of the E-nose device (DAQ, data acquisition system).

During the sampling step, air (a reference gas) flow from reference connector to valve 1 pass through the sample container to valve 2 and entering the gas sensor array setup. Meanwhile, during the delay or purging phase, the air flow from the reference gas directly passes through the gas sensor array. A 16-bit ADC in an Arduino Mega microcontroller was used for the data acquisition system.
Every second, a dataset with 10 signal values is sent from the microcontroller unit to the data logger using RS-232 serial communication. Prior to measuring, the E-nose was turned for about 30 min to ensure that a steady state response is obtained. Afterward, the configuration of the phase time was set as: 10 sec delay phase, 60 sec sampling phase, and 300 sec for purging phase. The assays were performed in-situ, using pure Ganoderma culture, compared with other soil fungi; and also oil palm plants-infected Ganoderma in the field in 4 levels of infection, i.e. healthy, mild, moderate and severe infection. The typical of sensor signal characteristics of the E-nose sensors is illustrated in Fig. 2, representing the 3 stages of the E-nose analysis (the delay phase, the sampling phase, and the purging phase) of the i-gas sensor for a j sample.

![Figure 2. Typical response of i-gas sensor in the E-nose when detecting j-sample.](image)

### 2.2. E-nose raw signal profiles and data pre-processing

The data matrix used for the statistical multivariate analysis included the E-nose signal profiles gathered by the eight MOS gas sensors, during the tea samples analysis. The matrix data of each tea sample contained 8 sensors × 3 700-row data. Each i sensor of the E-nose, an electrical signal profile was generated over the analysis time-period, for each j sample (Vij(t)). The time-dynamic response of each sensor depends on several physical parameters, such as flow rate in the sampling system, the sample’s headspace, the reaction time between the sample volatile compounds and the sensing material, and the environmental conditions like pressure, temperature and humidity. In this work, 3 types of data pre-processing techniques were applied namely the time set value, the area under the curve (as illustrated a shading area in Fig. 1), and the maximum value \( \max(V_i(t)) \) pre-processing tools. When required, a time-period of 70 sec was used, corresponding to the time delay and the sampling analysis time as illustrated in Fig. 1.

### 2.3. Statistical analysis using unsupervised and supervised pattern recognition tools

The dataset included the pre-processed signals gathered by the 8 MOS gas sensors comprised in the E-nose during the assays corresponding to independent samples evaluated. Principal component analysis (PCA) was used as an unsupervised pattern recognition method aiming to reduce experimental data dimension. So, PCA would allow a preliminary evaluation of the capability of the E-nose to be used as an unsupervised classification model of the tea samples according to their quality level.

The E-nose performance for quality assessment, was further evaluated by applying 4 supervised statistical methods, namely LDA, QDA and SVM with linear and radial kernels (RBF kernel).
Previously to the statistical analysis, the data matrix was subjected to a normalization procedure (scaling and centering techniques). After this normalization step, the initial database was split into 2 groups, the training data subset and the testing data subset. The training data subset was used to establish the classification model, being selected the one that allowed achieving the best classification performance for the repeated K-fold cross-validation (CV) procedure (10 repeats × 10 folds, which ensured that at each validation run, 10% of the training data was left for internal-validation purposes), while the testing data subset was used for the external-validation (full prediction) of the classification model previously established. The overall modeling development and analysis were performed using the open-source statistical software R (version 3.5.1) and using the caret, MASS, and kernel libraries.

3. Results and discussion

Generic E-nose usually cannot be used for every application. In this initial activity as an introduction E-nose was assembled using 8 sensors, namely: TGS 822, 832, 826, 2620, 2603, 2600, and 2612. Testing in the laboratory for Ganoderma mycelium when compared with other fungi (Trichoderma, Aspergillus and Omphalina) showed significant differences when analyzed using the unsupervised PCA chemometric system (Fig. 3). Tests for pathogenic Ganoderma isolates (Rejosari and CSB) compared to non-pathogenic Ganoderma lucidum, showed that Ganoderma was clustered with linear discriminant analysis (LDA) analysis in almost the same quadrant (Fig. 4). This showed that using E-nose could distinguish Ganoderma from other fungi (non-Ganoderma), even from non-pathogen.

E-nose data processed using machine learning Support Vector Machine (SVM) was able to distinguish the aroma between G. boninense CSB, G. boninense ‘Rejosari’, and G. lucidum with an accuracy rate of 99.64%. A clearly separation occured between the sample of the infected and healthy

![Figure 3. Results of Principal Component Analysis (PCA) of Ganoderma and other fungi.](image1)

![Figure 4. Results of Linear Discriminant Analysis (LDA) of Ganoderma CSB isolates (red), G. lucidum (green), and Ganoderma ‘Rejosari’ (blue).](image2)
root and stem (Fig. 5). Healthy samples are scattered in positive and negative areas while sick samples clustered in negative areas.

**Figure 5.** Principal Component Analysis (PCA) results of oil palm nurseries on the roots, stems and leaves of healthy and *Ganoderma*-infected plants.
E-nose was able to distinguish the stem with 4 categories infection (healthy, early, moderate and severe) with high accuracy (97.1%). There was 1 stem sample with a condition of early infection (actual) identified (predicted) as moderate infection and vice versa (red arrow). In general, healthy stem samples were very distinct from stems in a diseased condition (Fig. 6).

![Figure 6. Linear Discriminant Analysis (LDA) of 4 categories of infected stems (early, moderate, severe and healthy).](image)

E-nose was also able to differentiate leaves with 4 categories infection (healthy, early, moderate and severe) with high accuracy (89.62%). There was one sample with a condition of early infected (actual) recognized (predicted) as a leaf severe infected (red arrow) (Fig. 7).

![Figure 7. Linear Discriminant Analysis (LDA) of 4 categories of infected leaves (early, moderate, severe and healthy).](image)

E nose was also competent in distinguishing roots with 4 categories infection (healthy, early, moderate and severe) with high accuracy (90.95%) of each level of infected even though there was still a slice between early and moderate infected root (red arrow) (Fig. 8). E nose was able to discriminate soil with 4 categories sample with high accuracy (87.5%). Each soil sample with each actual condition was identified as it is in reality, although some soil samples from early and moderate infected were difficult to distinguish (Fig. 9).
4. Conclusion

*Ganoderma* mycelium when compared with other fungi (*Trichoderma, Aspergillus* and *Omphalina*) showed significant differences when analyzed using an unsupervised PCA chemometric system. The E-nose data processed using machine learning Support Vector Machine (SVM) was able to distinguish the aroma between *G. boninense* CSB, *G. boninense* ‘Rejosari’, and *G. lucidum* with an accuracy rate of 99.64%. E-nose was able to differentiate with high accuracy (90.95%) of each infection level even though there was still a slice between in root sample.

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