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Volatile Compound Detection of Banana Anthracnose Caused by *Colletotrichum musae* Using Electronic Nose

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ABSTRAK/ABSTRACT

Deteksi senyawa volatile pada penyakit antraknosa pisang yang disebabkan oleh *Colletotrichum musae* menggunakan hidung elektronik

Deteksi dini penyakit tanaman merupakan salah satu strategi pengelolaan penyakit pada tanaman sehingga tindakan pengendalian dapat diterapkan tepat waktu. Kebusukan pada buah adalah salah satu target penyakit pasca panen yang perlu dikelola. Metode deteksi dini penyakit tanaman di antaranya dapat dilakukan dengan memanfaatkan teknologi *electronic nose* (hidung elektronik). Penelitian ini menguji kemampuan hidung elektronik untuk mendeteksi keberadaan *Colletotrichum musae*, jamur penyebab busuk buah antraknosa pada pisang menggunakan sensor gas dengan mendeteksi konsentrasi senyawa volatil. *C. musae* diidentifikasi secara visual dan mikroskopis. Metode *detached-fruit assay* digunakan dalam percobaan ini. Suspensi jamur konidia pada konsentrasi $2{,}5 \times 10^6$ konidia/ml disuntikkan ke dalam sembilan replikasi sampel pisang homogen, sementara sembilan sampel lainnya disuntik dengan akuades steril yang berfungsi sebagai kontrol negatif. Informasi yang diperoleh dari hidung elektronik dianalisis menggunakan analisis komponen utama. Hasil penelitian menunjukkan bahwa dari enam sensor gas yang digunakan pada *electronic nose*, sensor gas MQ-4, MQ-5, dan MQ-8 mampu mendeteksi perbedaan yang signifikan antara pisang terinfeksi bila dibandingkan dengan kontrol dengan nilai konsentrasi sebesar 166,83; 173,02; dan 87,40 ppm pada pisang yang terinfeksi *C. musae* dan 20,90; 0,01; dan 44,97 ppm pada kontrol. Dengan demikian, ketiga sensor ini dapat digunakan sebagai acuan untuk mengembangkan teknologi dini dalam mendeteksi penyakit antraknosa pada pisang.

Keywords:
Colletotrichum musae, Fruit rot, Gas sensor, Internet of Things, Metal oxide

Early detection of plant diseases is one of the strategies for managing diseases in plants so that control measures can be implemented in a timely manner. Fruit rot is one of the targets of post-harvest diseases that need to be managed. Early detection methods of plant diseases can be carried out by utilizing electronic nose technology. This study examined the electronic nose capacity to detect the presence of *Colletotrichum musae*, the causative agent of anthracnose fruit rots on bananas using gas sensors by detecting the concentration of volatile compound. *C. musae* was identified visually and microscopically. Detached-fruit assay method was used in this experiment. A conidial fungal suspension at a concentration of 2.5×10^6 conidia/ml was injected into nine replications of homogenous banana samples, while nine other samples were injected with sterile water served as negative controls. The information obtained from the electronic nose was analyzed using principal component analysis (PCA). The results showed that from six gas sensors used in the electronic nose, MQ-4, MQ-5, and MQ-8 were the gas sensors that have detected significant difference between the infected banana and the control with a concentration value of 166.83, 173.02, and 87.40 ppm in banana infected with *C. musae* and 20.90, 0.01, and 44.97 ppm in controls. Therefore, these three sensors can be used as a reference for developing methods for detecting anthracnose disease in bananas.

INTRODUCTION

Banana production has undergone tremendous changes in the last century to meet the increase in worldwide demand for one of the most popular fruits. Originating from Southeast Asia, bananas are now grown in at least 135 countries worldwide in tropical and subtropical regions. This increase in production is centered largely around one variety, it is Cavendish banana which leads the world in both international trade of the fruit via large-scale monoculture plantations, as well as serving as a significant player in local markets and providing over 40% of global production (Drenth & Kema, 2021).

One of the major problems in the banana industry is the control and management of diseases. Most of them are of fungal origin, which can affect the plant and reduce the yield, quality, and shelf life of the fruit. Fruit rot is one of the most important diseases in banana fruit, caused by fungi as *Colletotrichum musae*. It is the most important disease of bananas in the postharvest. The losses of banana fruit yield caused by *C. musae* aroud 20-25% during postharvest (Filho *et al.*, 2021).

C. musae, as the causal agent of anthracnose, is a destructive postharvest disease in bananas (Qiao *et al.*, 2022). *C. musae* causes anthracnose in banana by showing black dot that can develop to be water-soaked spots, red orange colored with irregular shapes, and sunken or growing lesion (Saurabh *et al.*, 2023). Therefore, rapid, accurate, and low-cost detection of pathogens are essential to reduce the level of losses due to post-harvest disease (Ali *et al.*, 2023).

Current methods for detection and quantification of microorganisms, including isozyme analysis, vegetative compatibility group (VCG) analysis, and enzyme-linked immunosorbent assay are often expensive to implement and time consuming to perform (Dayarathne *et al.*, 2020). Therefore, quicker and more effective techniques are needed. As a result of pathogen attacks, plants release some volatile organic compounds (VOCs) which reflect their health and physiological conditions and are ideal indicators for non-invasive disease monitoring (Steglinska *et al.*, 2022). The detection of plant pathogens with VOCs as detected by VOC analysis. In this respect, gas chromatography-mass spectrometry (GC-MS) methods had been typically employed for the VOCs monitoring (Lim *et al.*, 2023). Electronic Nose appears to be the proper technique for the VOCs detection as parallel many alternatives because it is quick, less invasive, cheap, and straight forward application.

An electronic nose is an electronic odor detection system that works by imitating the way human nose recognizes odors through its receptors (Kim *et al.*, 2022). In an electronic nose, the function of these receptors is replaced by an array of gas sensors called a sensor array (Astuti *et al.*, 2022). Each electronic nose is uniquely designed for a specific purpose, as it uses a different combination of gas sensors and detects different types of odors. Currently, various types of metal oxide semiconductor (MOS)-based sensors, such as the Figaro TGS and MQ series, are widely available in the market at quite affordable prices (Wolfrum *et al.*, 2006; Shiddiq *et al.*, 2021a). This opens opportunities to build cost-effective electronic nose systems. Furthermore, the TGS and MQ series sensors are now also available in modular form, making the process of designing an electronic nose system simpler.

Electronic nose systems have been widely developed for the agricultural sector, especially for evaluating the quality of fruits and vegetables based on the aroma produced by the fruits and vegetables. Electronic noses have been used to monitor the ripeness level of fruits (Hendrick *et al.*, 2022), classifying the aroma of green tea (Kusairi *et al.*, 2022), and evaluating the quality of honey (Shiddiq *et al.*, 2021b). Although much research has been done, the use of electronic noses to detect anthracnose disease in bananas has not been done.

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Therefore, this study aims to detect anthracnose disease in bananas using electronic nose by utilizing several gas sensors.

MATERIAL AND METHODS

Preparation of *C. musae* Conidia Suspension

C. musae was isolated from banana showing anthracnose disease. The isolation was conducted at the Laboratory of Plant Protection Biotechnology, Faculty of Agriculture, Universitas Padjadjaran following method by Agrios (2005). The fungus was grown on Potato Dextrose Agar (PDA) media (200 g potato, 20 g dextrose and 20 g agar in 1 l RO water). Conidia were harvested using a loop syringe and mixed with 10 ml of sterile distilled water. The conidial density was calculated using a hemocytometer. Serial dilution was performed to achieve a conidial density of 10^6 conidia/ml if the conidial density was higher than expected (Mukhopadhyay *et al.*, 2025).

Preparation of Banana Samples

Nine banana samples were used during this research. The bananas used in this study were in a fully ripe condition, characterized by a smooth yellow peel without brown spots. Before the inoculation, banana samples were surface sterilized using 70% alcohol for 15 seconds then the banana samples were rinsed using sterile distilled water and dried by tapping gently the banana using sterile tissue paper. The center of each banana sample was pricked using a needle. Each banana sample was inoculated with 10 µl conidial suspension of *C. musae*. The samples used as control were injected with 10 µl sterile distilled water (Silva *et al.*, 2021). The incubation was done by hanging the bananas in a closed space at room temperature.

Volatile Compound Measurement using Electronic Nose

Samples were labelled and stored in a closed space at a temperature of 26°C for 7 days. The inoculated banana samples were examined using a prototype electronic nose device with six gas sensors. The selection of gas sensors in this experiment was based on previous research that examined the detection of anthracnose disease in tomatoes using a prototype of electronic nose (Khlaif *et al.*, 2024). The details about gas sensors used in this experiment are shown in Table 1 (Khlaif *et al.*, 2024). The gas sensor is assembled using Arduino UNO R3 into an electronic nose. The sketch and the work principle of the electronic nose in measuring volatile compound gases is described in the following image (Figure 1).

Table 1. The details about gas sensors in *Electronic Nose* used in this study

Sensor Type	Gas Target	Detected Compounds (ppm)
MQ-3	Alcohol Vapor	10-300
MQ-4	Natural Gas and Methane	CH_4 dan Natural Gas (200-10,000)
MQ-5	LPG, Natural Gas and Coal Gas	LPG, Liquid Natural Gas, Natural Gas, Isobutana, Propana, and Town Gas (200–10,000)
MQ-6	LPG, Propane	LPG, Liquid Natural Gas, Isobutane, Propane, (200-10,000)
MQ-8	Hydrogen	100-10,000
MQ-135	Air Quality Control (NH3, Benzene, Alcohol and Smoke)	10-10,000

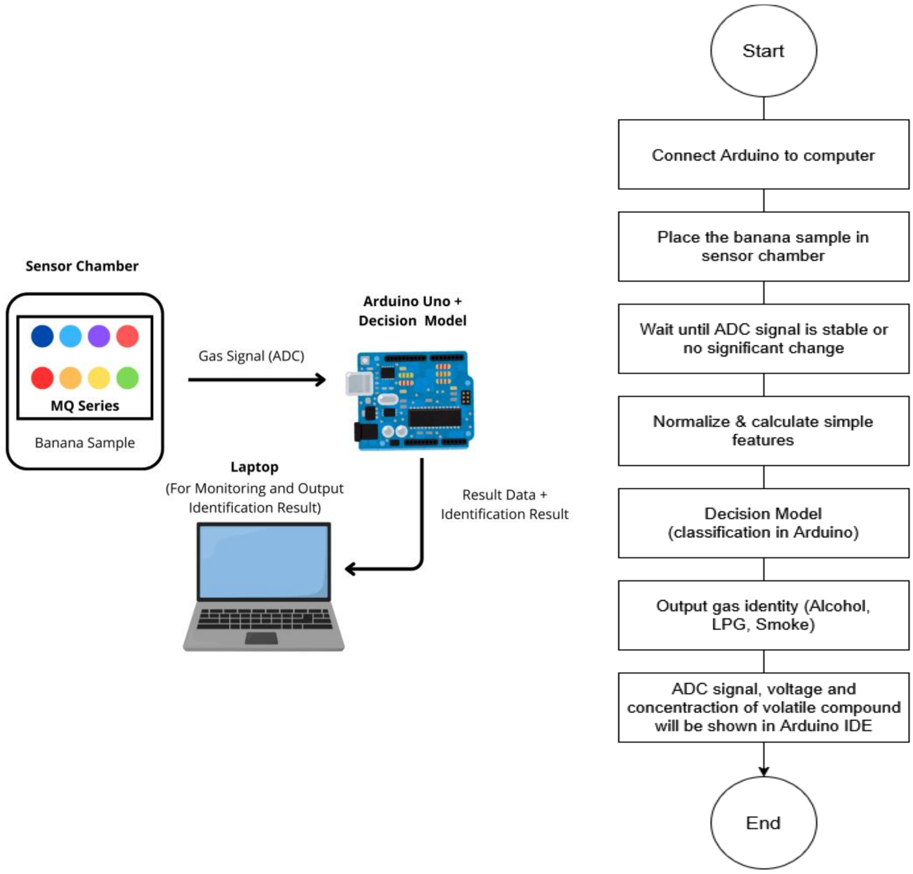

Figure 1. Sketch and workflow of electronic nose system development

Principal Component Analysis

The ability of gas sensors in electronic nose to distinguish between banana infected with *C. musae* and control samples was assessed with PCA score plot for the observed electronic nose ADC signal values (which represent the sensor output voltage) and volatile compound concentration using SPSS 26 (Khlaif *et al.*, 2024).

RESULTS AND DISCUSSION

Identification of *C. musae*

The findings obtained from the morphological identification of *C. musae* showed that there were sunken or black lesions, indicating a moderate to severe level of disease (30-50% of the peel area affected). Orange spots were observed around the sunken lesions which are conidiomata. Conidiomata grow as clumps containing conidia until it splits irregularly to release the conidia and sometimes opens wide in several species of *Colletotrichum*. The conidia exhibited a straight and has fusiform shape with acute ends (Saurabh *et al.*, 2023). The visuals of banana sample as inoculant and microscopic identification results are shown in Figure 2.

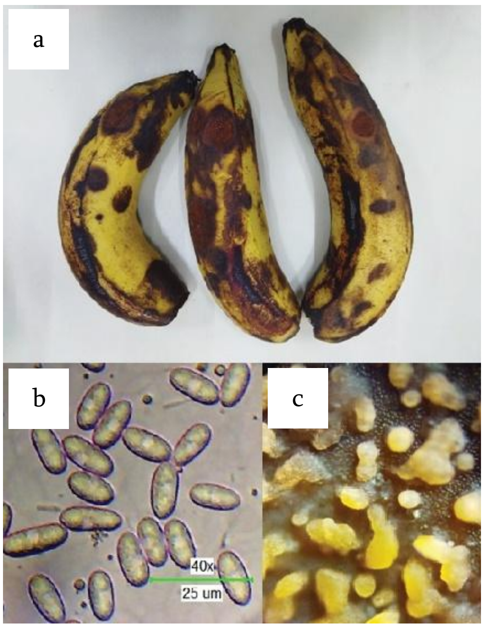

Figure 2. Identification of *C. musae*. a) Banana fruit infected by *C. musae*; b) Conidia of *C. musae* in 40X magnification; and c) Conidiomata of *C. musae*.

Principal Component Analysis

Out of six gas sensors used in the electronic nose, three gas sensors had a good response in detecting the existence of anthracnose disease in bananas, they are MQ-4, MQ-5, and MQ-8 sensors. The ADC data obtained from the Arduino microcontroller were recorded as a time series and then averaged to minimize noise. The ppm values were derived from the conversion of the sensor response data based on the calibration curve, rather than measured directly using a separate ppm measuring instrument. The value of ADC signal, voltage, and volatile compound concentration is shown in Table 2.

Table 2. The values result of ADC (Analog to Digital Converter) signal, voltage, and volatile compound concentrations measurement using electronic nose.

Sensor	ADC Signal	Voltage	Volatile Compound Concentration (ppm)	Sensing Resistance	Reference Resistance
MQ3-c	644	3.15	6.10	5.89	6.61
MQ3-i	711	3.48	29.27	4.39	9.75
MQ4-c	479	2.34	20.90	11.36	2.82
MQ4-i	568	2.78	166.83	6.34	4.22
MQ5-c	330	1.61	0.01	21.00	0.97
MQ5-i	626	3.06	173.02	6.34	3.87
MQ6-c	824	4.03	6.14	2.42	0.24
MQ6-i	820	4.01	6.16	2.48	0.24
MQ8-c	614	3.00	44.97	6.66	0.08
MQ8-i	740	3.62	87.40	3.82	0.11
MQ135-c	323	1.58	1.82	21.67	4.25
MQ135-i	388	1.90	12.49	16.37	6.99

Note: c means control and i means inoculated (treatment), since temperature and RH data were not recorded due to the absence of supporting sensors, this remains a limitation of the present study.

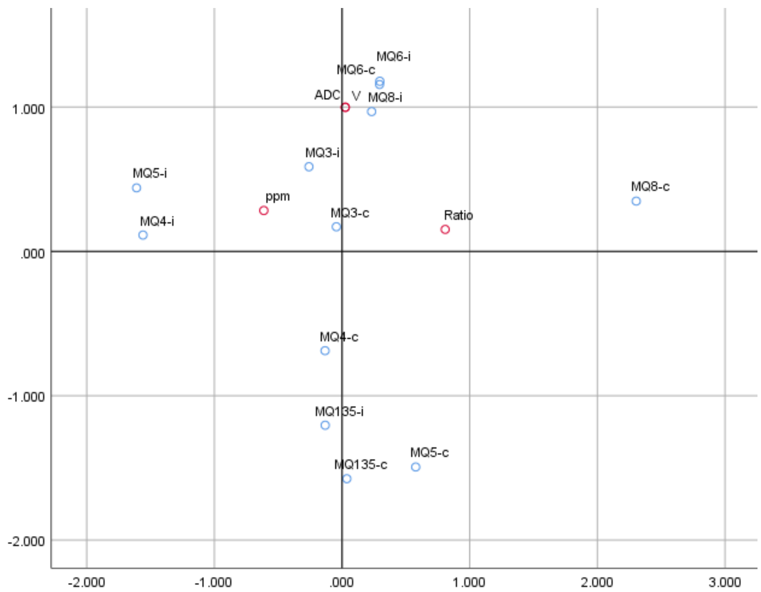

Figure 3. Principal component analysis for banana samples 7 days after inoculation with *C. musae* using electronic nose.

MQ-5 had the highest difference between bananas infected with anthracnose and controls with a concentration value of 173.02 ppm in banana inoculated with *C. musae* and 0.01 ppm in controls. Meanwhile, the MQ-4 and MQ-8 sensors had compound concentration values of 166.83 ppm and 87.40 ppm respectively in bananas inoculated with *C. musae* and 20.90 ppm and 44.97 ppm in the control.

Other sensors used in this experiment such as MQ-3, MQ-6, and MQ-135 have no significant difference between the inoculated bananas and the control. PCA was not implemented in the microcontroller. The sensor output data read by Arduino was transferred to the computer for further analysis. Principal Component Analysis (PCA) was performed using SPSS after the data collection. Thus, the microcontroller only functioned as a data acquisition device, while the PCA-based identification process was carried out on the computer. The result of analysis using PCA is shown in Figure 3.

The score plot of principal component analysis for the seven days after inoculation is represented in Figure 3. The PCA score plot showed that a clear separation between healthy and inoculated bananas was observed on the seventh day after inoculation. At this stage, the bananas already exhibited visible symptoms such as sunken or black lesions, indicating that the electronic nose confirmed the disease but was not able to distinguish it earlier when the fruits were still visually similar to the healthy ones. The outcomes of principal component analysis indicated that electronic nose successfully distinguished between control and infected samples. The signs (a few dark, small, sunken spots with a water-soaked look) of fungal development were apparent seven days post-inoculation (Singh *et al.*, 2021) (Figure 4).

Figure 4. Banana inoculated with *C. musae* after seven days and the control (the inoculation spot pointed by the red arrow).

The distance between each gas sensors represents the difference between them. As the result of volatile compounds using electronic nose, the points of MQ4, MQ5, and MQ8 between the inoculated banana (i) and control (c) has the furthest distance. Meanwhile, MQ6-c and MQ6-i do not have a distance because the compound values from measurements using the electronic nose do not differ significantly, so the points overlap. The difference in the location of the plot scores is influenced by the difference in analog signals produced by each sensor. The compounds that can be detected by each gas sensor and the high concentration of compounds volatilized by bananas also cause differences in the distance between plot scores.

With the high difference between the compounds produced by bananas infected with *C. musae* and the control on the MQ-4 and MQ-5 sensors, natural gas is one of the compounds produced by *C. musae* infection in bananas. Excretion and accumulation of natural gases during *C. musae* infection in banana fruit is the main factor causing anthracnose symptoms (Khlaif *et al.*, 2024; Berhal *et al.*, 2017). These results are in accordance with the measured values of two of sensors used in the data analysis, they are MQ-4 and MQ-5 which are related to natural gas (Mukhtarov *et al.*, 2024; Sakayo *et al.*, 2019).

With the high difference in ADC signals obtained between bananas inoculated with *C. musae* and controls on the MQ-5 sensor, this study shows that the electronic nose equipped with a MOS sensor has the potential to detect the presence of anthracnose disease in banana. Thus, these findings indicate that electronic nose devices can be developed as early warning systems in plantations and fruit storage facilities. In another study, potato brown rot caused by *Ralstonia solanacearum* was detected by electronic nose and PCA analysis. Electronic nose with MOS gas sensors was used in the detection of different stages of Lethal Bronzing Disease (LB) in infected cabbage palms (*Sabal palmetto*) (Biondi *et al.*, 2014). VOCs type and concentration were detected using electronic nose and MOS sensors, in order to determine the stage of crop diseases and insect pests (Zheng & Zhang, 2022).

For the purpose of classifying complicated systems like water and food samples, devices that rely on electronic language (such as electronic nose, electronic tongue, and electronic eye) are taken into account when incorporated into the input management system. An electronic nose device was used to identify and classify fungal early infections on garlic where the fungal infections were caused by *Fusarium oxysporum* f. sp. *cepae* (FU), *Alternaria embellisia* (syn. *Embellisia allii*) (AL), and *Botrytis* *allii* (BO) (Li *et al*., 2010). PCA analysis and further MVDA techniques were used to examine the resulting electronic nose data. Advances in diagnostic technologies are facilitated by the development and refinement of novel electrical and operational techniques related to the chemical detection of complicated gas mixtures that contain volatile organic compounds (VOCs). In the future, these gadgets might be utilized as alarm systems for several agricultural and biological applications.

CONCLUSION

The result of this experiment showed that electronic nose using MOSs Sensor can detect volatile compounds in banana fruit infected with anthracnose disease. The gas sensor that showed the detection of volatile compounds that had a significant difference with the banana inoculated with *C. musae* and the control was MQ-4, MQ-5, and MQ-135 so this gas sensor can be used as a reference to detect anthracnose disease in bananas. Specifically, at day 7 after inoculation, the average ADC and ppm values of several sensors showed significant differences between healthy and infected samples. For instance, volatile compound detected by MQ4 increased from 479 (≈20.90 ppm) in healthy bananas to 568 (≈166.83 ppm) in infected bananas, by MQ5 rose from 330 (≈0.01 ppm) to 626 (≈173.02 ppm), and by MQ8 from 614 (≈44.97 ppm) to 740 (≈87.40 ppm). These quantitative differences correspond to the visible symptom level (sunken and black lesions), confirming that these gas sensors can serve as an effective reference for anthracnose detection.

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