الكشف عن فطر *Colletotrichum coccodes* المسبب لعفن الفاكهة (anthracnose) في البندورة باستخدام الأنف الإلكتروني

إعداد

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Detection of *Colletotrichum coccodes* causing anthracnose fruit rots in tomato using electronic nose

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I hereby declare that this thesis Titled: "Detection of *Colletotrichum coccodes* causing anthracnose fruit rots in tomato using electronic nose" is the product of my own efforts, except what has been referred to, and this thesis as a whole or any part of it has not been submitted as a requirement for attaining a degree or scientific or research title to any other educational or research institution.

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Dedication

Firstly, I dedicate this achievement to my father's soul: Wael Khalif, whose words of encouragement did not leave me for a moment. Despite the absence of his body, his spirit is still present. Also, I would like to dedicate this work to my loving husband: Muhammad Shahwan for his overwhelming and endless support until the last moment. As well as my mother: Thikra Sleem my guiding angel, whom I would not have reached this level without her presence in my life, to the light of my life, my daughters: Meso and Hamsa. Last but not least, to my dear sisters: Shaza, Sherin, Rana, and Bessan, and my loving brother: Mohammad for every kind and encouraging word.

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Abbreviations

Abbreviations	Full Name
%	Percent
/ml	Per Millimeter
˚C	Celcius Degree
µl	Microliter
µM	Micrometer
AFLP	Amplified Fragment Length Polymorphisms
ANN	Artificial Neural Networks
BLASTn	Basic Local Alignment Search Tool- Nucleotide
bp	Base pairs
C. coccodes	*Colletotrichum coccodes*
CA	Hierarchical Cluster Analysis
Cat #	Cataloge Number
cDNA strands	Complimentary DNA strands
cm	Centimeters
CP	Conducting Polymer
DDW	Double-Distilled Water
dH_2O	distilled water
DNA	Deoxyribonucleic acid
DNase free water	Deoxyribonuclease free water
DW	Distilled Water
EC	Electrochemical
EDTA-TBE	Tris-borate-ethylenediaminetetraacetic acid
e.g.	For Example
EN	Electronic Nose

ELISA	Enzyme-Linked Immunosorbent Assay
EN	Electronic Nose
et al.	And Others
etc	And The Rest
FAOSTAT	Food and Agriculture Organization Statistics
g	Gram
GC-MS	Chromatography-Mass Spectrometry
ha	Hectare
hr	Hour
i.e	That Is
kbp	Kilobase-Pairs
LB	Lethal Bronzing Disease
LDA	Linear Discrimination Analysis
$MgCl_2$	Magnesium Chloride
Mha	Million Hectares
min	Minutes
ml	Milliliter
mm	Millimeters
MOS	Metal-Oxide Sensors
MOSFET	Metal-Oxide Semiconductor Field-Effect Transistor
MVDA	Multivariate Data Analysis
NaOCl	Sodium Hypochlorite
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCs	Principal Components

PDA	Potato Dextrose Agar
PLS	Partial Least Squares
PLS-DA	Partial Least Squares-Discriminant Analysis
PN	Product Number
psi	Pound Per Square Inch
QCM	Quartz Crystal Microbalance
Taq-Man PCR	Quantitative Real-Time
™	Trademark Symbol
RAPD	Random Amplified Polymorphic DNA
rpm	Round Per Minutes
sec	Seconds
TM	Trademarke
USDA	United State Department of Agriculture
UV	Ultraviolet
VCG	Vegetative Compatibility Group Analysis
VOCs	Volatile Organic Compounds

Abstract

Detection of *Colletotrichum coccodes* causing anthracnose fruit rots in tomato using electronic nose

Colletotrichum coccodes are the most known *Colletotrichum* isolates to be hard to control. Once the tomato gets infected with *C. coccodes*, the anthracnose develops within a few days. Therefore, early detection of the fungal infection is vital for successful prevention and effective treatment of the infected fields. This study aimed to employ the electronic nose (EN), with metal-oxide sensors (MOS), to sense the presence of *C. coccodes* on tomato fruits at different stages and concentrations.

Identification of *C. coccodes* was carried out using the polymerase chain reaction (PCR), where the identification of *C. coccode* fungus was achieved using three different primer sets: ITS1F/ITS4, Cc1F1/Cc2R1, and Cc1NF1/Cc2NR1. In which ITS1F/ITS4 was a general primer set for most *Ascomycetes* and *Basidiomycetes*. Cc1F1/Cc2R1 primer set was a specific primer to *Colletotrichum* spp., which gives a 447 bp product, and Cc1NF1/Cc2NR1 was a unique primer to *C. coccodes*, which results in a 349 bp product. In addition, three homogeneous tomato samples were injected with three different concentrations: $30*10^3$, $30*10^4$ and $30*10^5$ conidial fungal suspension, three other tomatoes were used as negative controls, and all were measured using an EN device every two days for ten days.

Specific primers (ITS1F/ITS4, Cc1F1/Cc2R1, and Cc1NF1/Cc2NR1) could recognize and specify *C. coccode* fungus using a suitable PCR program and sequencing.

The data acquired from the EN sensors were analyzed using principal component analysis (PCA). It was found that the EN device could sense pathogen occurrence at early stages, *i.e.* after two days of the infection, and before the appearance of any symptoms, where the PCA scores plot explained 100% of the total variance. In addition, the overall scores plot explained 100% of the total variance indicating the ability of EN to differentiate between different stages of infection duration (*i.e.* second, fourth, sixth, eighth, and tenth day). Moreover, the concentration of the infected samples was clearly differentiated on the sixth, eighth and tenth days.

Keywords: Electronic Nose, anthracnose fruit rots, *Colletotrichum coccodes*, multivariate data analysis.

Chapter One: Introduction

1. Introduction

1.1 Background

Solanum lycopersicum L. (*Solanaceae* family), artificially known as Tomato, is a major popular cultivated crop up to this moment. In the past decade, the production and cultivation of this crop have increased fivefold (Alkowni *et al.*, 2019). In Palestine, it is considered one of the most important crops in the fruit sector, in which the estimated planted area with tomatoes is about 1200 ha. Also, it constitutes about 1.5% of the total cultivated agricultural land in the country (Al-Tardeh and Iraki, 2013; Najjar, 2020).

However, this crop is vulnerable to several pathogenic microorganisms such as *Colletotrichum* spp., which is recorded as the eighth-most significant group of plant pathogenic fungi wild world, according to the perceived economic and scientific importance (Liu *et al.*, 2011; Dean *et al.*, 2012). These pathogenic fungi cause anthracnose and other diseases on the leaves, stems and fruits of several plants. These diseases include black dot, tuber blemish and foliar disease of potato. In addition to anthracnose on tomato fruits (Belov *et al.*, 2018). According to the United State Department of Agriculture (USDA) databases, *C. lycopersici* and *C. coccodes* were recorded as the most effective fungi affecting tomatoes. Furthermore, *C. coccodes* can affect 109 different plant species and even humans (Liu *et al.*, 2011; Testen and Miller, 2018).

Identification of a fungal pathogen can be achieved using numerous diagnostic and applicable techniques such as; isozyme analysis, vegetative compatibility group analysis (VCG) and enzyme-linked immunosorbent assay (ELISA). Moreover, nucleic acid-based techniques such as polymerase chain reaction (PCR) and Deoxyribonucleic acid (DNA) array technology have been

utilized for identification purposes and the gas sensor array technology (Ben-Daniel *et al.*, 2009; Narayanasamy, 2011; Abu-Khalaf *et al.*, 2018; Qneibi *et al.*, 2018; MacDougall *et al.*, 2022).

Over the past decades, electronic nose (EN) (which is a gas sensor) technology has achieved unlimited applications that targeted a wide range of study areas ranging from environmental monitoring to medical diagnostics and recently has appeared as an effective way to verify food spoilage (Concina *et al.*, 2009; Abu-Khalaf and Masoud, 2022). The discovery and improvements of new electronic and operating methods correlated with chemical detection of complex gas mixtures, which consist of volatile organic compounds (VOCs), are contributed to the progress in diagnostic techniques. In addition to automatic learning (*i.e.* artificial neural networks (ANN)), libraries and databases of disease references, data analysis programs and identification of disease biomarkers; these advances have brought considerable EN applications for detecting diseases related to different causes (biotic, abiotic and genetic) (Oates *et al.*, 2020; Najjar, 2020).

ENs consist of multiple sensor sets responsible for detecting many chemical components such as alcohol, ammonia or methane. Metal oxide semiconductor sensors (MOS) are one of the most commonly used sensors in ENs (Karakaya, Ulucan, and Turkan, 2020; Oates *et al.*, 2020).

1.2 Objectives

1. To detect *C. coccodes* in tomato using EN.

2. To differentiate between the different stages of *C. coccodes* in tomatoes using EN.

Chapter Two: Literature Review

2. Literature Review

2.1 Tomato crop importance and production

Tomato (*Solanum lycopersicum L.*), a *Solanaceae* family, originated in South America and is the second cultivated crop in the world after potato, according to the Food and Agriculture Organization Statistics (FAOSTAT). It has about 181 million tons of crop yields from 5 Mha. Tomato's major country producers are Turkey, Egypt, Italy, Spain, and Morocco in the Mediterranean (FAOSTAT, 2019). In Palestine, tomatoes are grown on an area of more than 1,200 hectares, constituting 1.5% of the total cultivated agricultural land in the West Bank, where the governorates of Jenin and Tubas are the largest tomatoes producers (Masri and Alkowni, 2021). Many pathogens alter the production of fresh and processed tomatoes, including fungi, bacteria, phytoplasmas, viruses and viroids (Panno *et al.*, 2021).

Tomato is more susceptible to many diseases due to their intensive selection and severe genetic bottlenecks, which arose from the low genetic diversity of cultivated tomatoes during evolution and domestication. Moreover, during the planting and post-harvest period, it can be affected by more than 200 diseases caused by various pathogens worldwide (Al-Tardeh and Iraki, 2013; Panno *et al.*, 2021).

One of the devastating fungal pathogens of tomatoes is *C. coccodes*, which Cause anthracnose on ripe fruits (Liu *et al.*, 2011).

2.2 *Colletotrichum coccodes* (*C. coccodes*)

Colletotrichum coccodes is a plant pathogenic fungus that has been reported worldwide in various hosts (Belov *et al.*, 2018). It is found mainly in the *Solanaceae* and *Cucurbitaceae*, especially damaging potatoes, tomatoes and other

Solanaceous weed species (Dauch *et al.*, 2003). *C. coccode* is variable in genetic terms (Ben-Daniel *et al.*, 2010). It is a potential biocontrol agent for *Abutilon theophrasti* (Cannon *et al.* 2007). This species colonizes the above-ground parts as well as the underground parts of the host plant. It was first described in 1833 on potato in Germany as *Chaetomium coccodes. Colletotrichum atramentarium* and *Colletotrichum phomoides* are synonyms to *C. coccodes* (Jamiolkowska *et al*., 2018).

Colletotrichum pathogens are known as ammonium secretors. *C. coccodes* virulence is associated with the amount of ammonium secreted as measured in tomato (*Solanum lycopersicum* cv. *Motelle*) fruit tissue necrosis (Alkan *et al.*, 2009). In addition, it is an intracellular colonizer pathogen that commonly feeds on living host cells before turning into necrosis (Dauch, 2006).

Colonies of *C. coccodes* grown on PDA media are circular, initially white and becoming gray with age, forming black spots on agar. Also, these colonies have white, cottony sparse aerial mycelia and abundant, black sclerotia that are evenly distributed on the agar surface (Lees and Hilton, 2003; Liu *et al*., 2011). *C. coccode* has special straight conidia with sharp ends and is often slightly constricted in the central part. It is a post-harvest pathogen because of its latent disease initiated before harvest and becomes active in posterior times (Cannon *et al.*, 2007).

Once the tomato gets infected with *C. coccodes*, the anthracnose develops within a few days (Ben-Daniel *et al.*, 2009). Infection with *C. coccodes* usually occurs in the growing season, but symptoms and signs of infection are often expressed in relatively late stages. Therefore, early detection of fungal infection

is vital for successful prevention and effective treatment of the affected fields (Ingram *et al.*, 2011).

Symptoms first appear as small, circular spots on the skin, and later they develop dark spots, which are the fungal spore-producing bodies. At this point, the decay has penetrated deeply into the tomato pulp. Spotted fruits often rot totally due to the attack of secondary fungi through spots of anthracnose (Gleason and Edmunds, 2005).

There are no specific fungicides or even chemicals effective against *C. coccodes* in vitro. Moreover, there are no effective measures to control *C. coccodes* (Cullen *et al.*, 2002).

2.3 Methods for plant disease detection

Recognizing symptoms and early detection of pathogens are essential for successful disease management (Panno *et al.*, 2021). A wide range of conventional and current diagnostic methods are applied to identify and quantify fungal pathogens (isozyme analysis, vegetative compatibility group analysis (VCG) and enzyme-linked immunosorbent assay (ELISA)). In addition, nucleic acid-based diagnostic techniques, polymerase chain reaction (PCR), DNA array technology, and gas sensor array technology are also utilized (Narayanasamy, 2011; Abu-Khalaf and Masoud, 2022).

2.3.1 Conventional methods

Conventional methods depend on visual symptom interpretation or culturing and isozyme analysis to identify the pathogen (Figure 1). The conventional methods' accuracy and reliability rely mainly on the person's experience and skills. The diagnosis that requires culturing can be time-

consuming and impractical when aiming for rapid results (McCartney *et al.*, 2003).

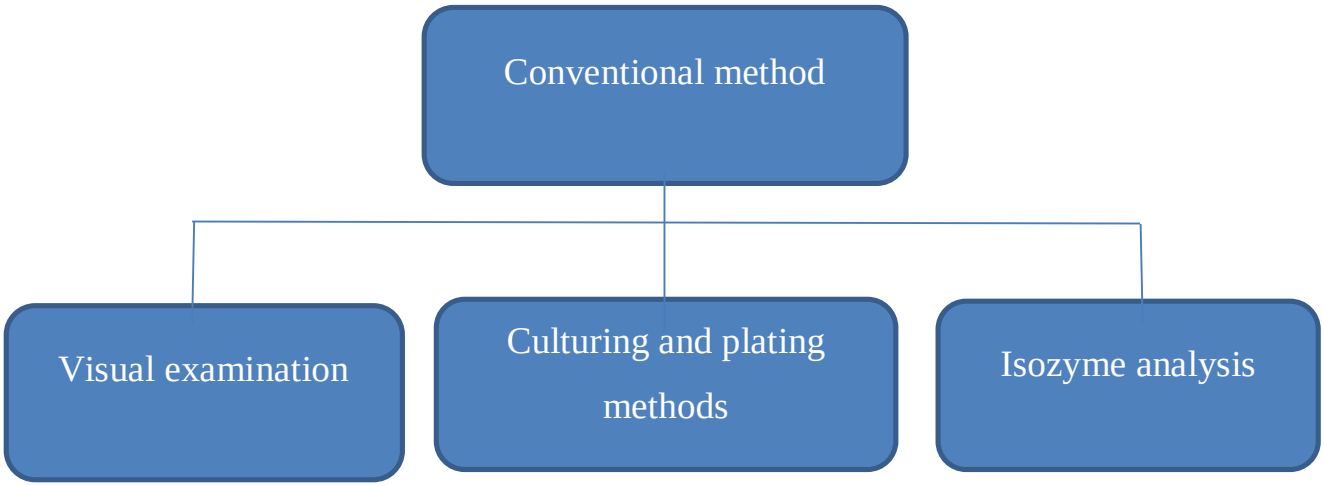


Figure 1: Conventional methods for the detection of a fungal pathogen (Ray *et al.*, 2017).

2.3.2 Current methods

Current methods are categorized into direct and indirect techniques (Figure 2). In which direct methods depend on laboratory experiments, mainly ELISA and PCR. On the other hand, the indirect techniques are advanced and based on imaging tools integration. These techniques depend on the incorporation of sensors and smart systems on-site to provide a more precise and rapid disease detection method (Rizk, 2017).

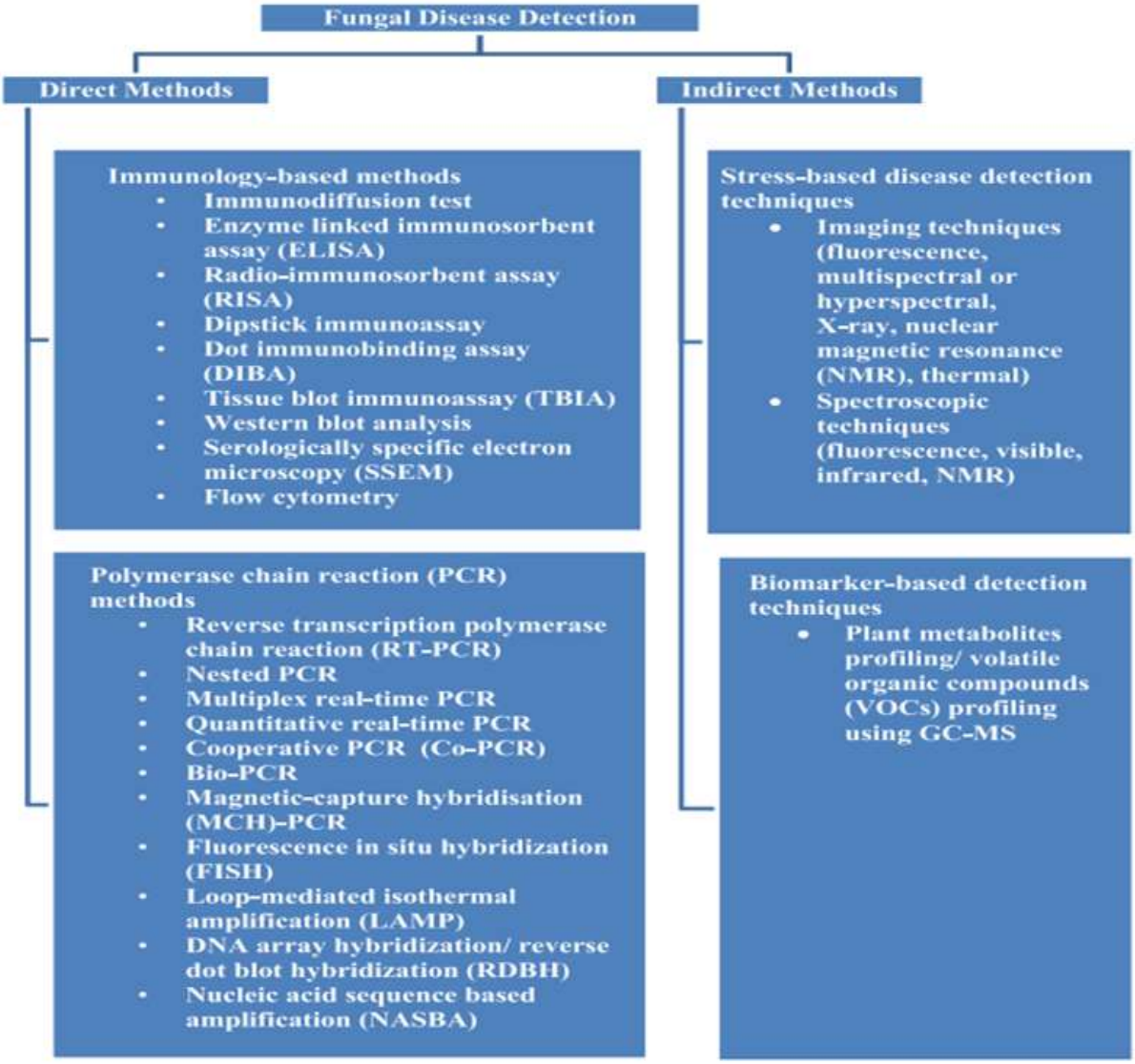


Figure 2: Current fungal pathogen detection methods (Ray *et al.*, 2017).

2.3.3 Polymerase chain reaction (PCR) method

Polymerase chain reaction (PCR) is a simple, rapid and sensitive technique where minute amounts of DNA are massively amplified by DNA polymerase into multiple copies using specific primer molecules (Schrader *et al.*, 2012; Garibyan and Avashia, 2013).

The amplification process is carried out through three stages that differ in temperature: the first stage is called denaturation, in which the DNA strands are separated by heating the PCR solution above the melting point of the target DNA. The second stage is called annealing, where the primers bind to the target DNA by lowering the temperature. The third stage is the extension that occurs by raising the temperature to allow the DNA polymerase to extend the primers (Garibyan and Avashia, 2013).

Molecular techniques have been adopted for their specificity and sensitive identification of large numbers of pathogens. In addition, the PCR method has relatively low operational costs and automatization. For instance, PCR and quantitative real-time (Taq-Man) PCR were used to detect and monitor *C. coccodes* on potato tubers and in soil using a specific set of primers (Cc1NF1/Cc2NR1) (Cullen *et al.*, 2002). Also, PCR-based techniques were used to detect *C. coccodes* on tomatoes and pepper infected artificially with the pathogen (Kim *et al.*, 2018).

Moreover, diagnostic molecular markers, generated from random amplified polymorphic DNA (RAPD), were used in PCR to detect the presence of *C. coccodes* on the target *Abutilon theophrasti* weed species (Dauch *et al.*, 2003). Also, amplified fragment length polymorphisms (AFLP) were used for the genetic characterization of *Colletotrichum* pathogens (O'Neill *et al.*, 1997).

However, molecular methods have a few limitations, such as primers' design and validation, in which each primer set has to be appropriate to a single, specific target, and the necessity of a sampling protocol. Moreover, this analysis is a destructive one and the economic value of sampled materials may be impaired by the sampling procedure (Cellini *et al.*, 2017).

2.3.4 Detection of plant diseases using sensors

The cornerstone of plant pathology is defined by early detection, rapid, precise and inexpensive diagnoses, which become essential for emerging diseases that have unobserved initial symptoms but are hard to be faced afterward (Buja *et al.*, 2021). Analysis of volatile organic compounds (VOCs) is an indirect method for detecting plant pathogens, in which these chemicals are considered as a defense mechanism against pathogen attack, which is produced and released by plants (Scala *et al.*, 2013).

As a consequence of the pathogenic infections, plants release certain VOCs, as a signal of their healthy physiological state and are therefore available for non-invasive monitoring of plant diseases (Cellini *et al.*, 2016; Al-Ramahi *et al.*, 2019).

VOCs are detected by gas chromatography-mass spectrometry (GC-MS) methods in a conventional way (Ewen *et al.*, 2004; Spadafora *et al.*, 2016; Abu-Khalaf and Haselmann, 2012), but they are complicated, time-consuming, expensive, massive, and need considerable practice for proper use (Sharma *et al.*, 2019).

Electronic nose (EN) appears to be the appropriate method for detecting VOCs among many alternatives as it is a fast, non-invasive, inexpensive, and easy method compared to GC-MS techniques option for many applications (Wilson, 2018).

2.4 Electronic Nose (EN)

EN is composed of an array of sensors that present different sensibilities to molecules belonging to several chemical classes. The electric signals from the sensors are elaborated to create a pattern corresponding to the gas composition

(Cellini *et al.*, 2017). Multivariate data analysis (MVDA) can be used to identify and quantify the analyzed samples (Mudalal *et al.*, 2020; Zaid *et al.*, 2020; Masoud *et al.*, 2021). There are various techniques for MVDA (e.g., partial Least Square (PLS), principal component analysis (PCA) and artificial neural network (ANN)) (Abu-Khalaf and Bennedsen, 2004; Abu-Khalaf and Abu Rumaila, 2020; Taha and Abu-Khalaf, 2020).

Expansion of methodologies for disease detection using EN devices entails a comprehensive understanding of the host's physiology and metabolic pathways affected by disease processes, diverse mechanisms of disease caused by biotic and abiotic diseases, and abnormal VOCs chemical classes released due to pathogenic effects on host metabolic processes (Wilson, 2018). However, each VOC chemical has its superior quality and properties. Therefore, digital signatures of chemicals, which will be the input data for the EN instrument, are specific. The sensor array is responsible for recognizing target chemicals in the medium, where every single sensor is responsible for sensing a particular type of aroma (Szulczynski and Gebicki, 2017; Taha and Abu-Khalaf, 2020; Oates *et al.*, 2020).

EN distinguishes between various gases by simulating the human nose, mimicking the biological olfactory system, where odor receptor cells are replaced with gas sensors and sensing materials. ANN, computing algorithms, and data analysis applications replace the neural network and brain (Figure 3) (Oates *et al.*, 2020; Karakaya *et al.*, 2020; Abu-Khalaf, 2021; Al-Mahasneh *et al.*, 2021; Abu-Khalaf and Masoud, 2022).

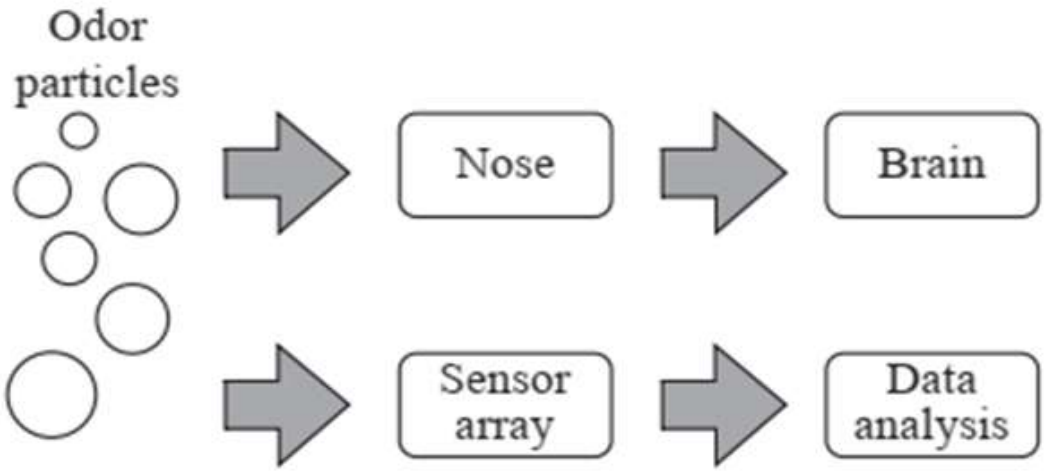


Figure 3: Similarities between the olfactory system and the EN technology (Karakaya *et al.*, 2020).

Choosing the appropriate sensors for a specific task is a core factor in determining the applicability of the electronic nose (Szulczynski and Gebicki, 2017). Sensor types that can be used include metal oxide semiconductor (MOS), quartz crystal microbalance (QCM), metal oxide semiconductor field-effect transistor (MOSFET), electrochemical (EC) and conducting polymer (CP) (Sanaeifar *et al.*, 2017).

2.4.1 Metal-oxide sensors (MOS)

MOS is the most used sensor type in EN devices. This is due to its convenience for an extensive number of gases (Karakaya *et al.*, 2020; Zheng and Zhang, 2022). The MOS operating principle is based on the variation in the conductivity of the oxide when interacting with the gas and the change is generally proportional to the gas concentration (Figure 4) (Hsieh and Yao, 2018).

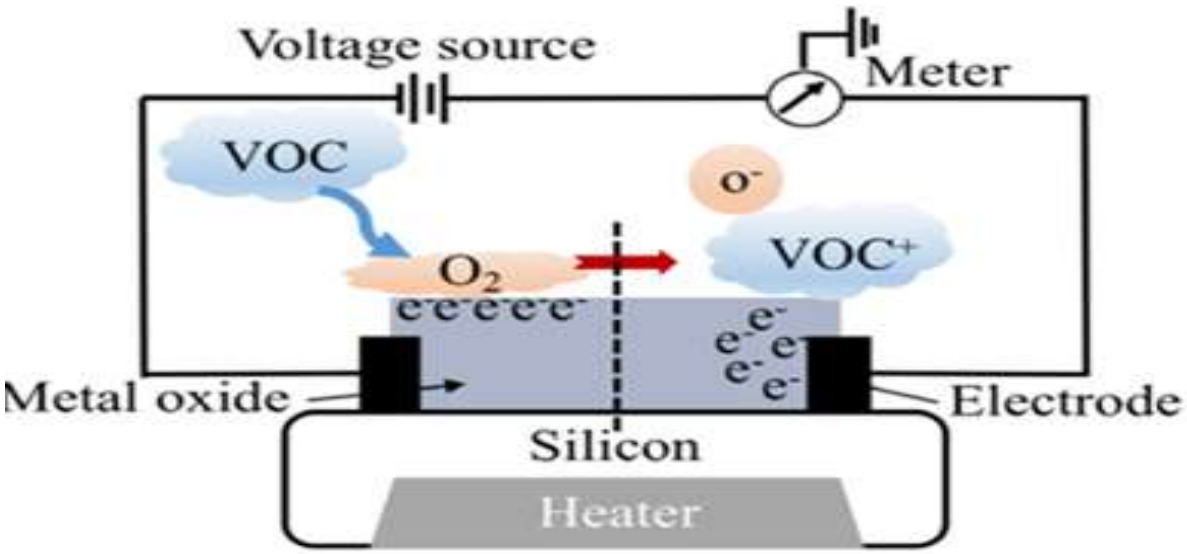


Figure 4: The working mechanism of metal-oxide sensor technology (Hsieh and Yao, 2018).

MOS are classified into two major groups according to their responses to various gases (Karakaya *et al.*, 2020). The first group is n-type. Its operational principle is based on the reactions between the oxygen molecules in the air and the sensor's surface. These reactions lead to the trapping of free electrons on the surface, resulting in potential intergranular barriers that prevent carrier movement, which produces large resistance regions. The second group is p-type. It reacts with oxidizing gases leading to electron removal and whole production (Kim and Lee, 2014).

2.4.2 Electronic Nose (EN) applications

EN portable system, combined with sensitive gas sensor arrays, is an innovative method that has some advantages over other technologies and addresses some challenges like field application (Cui *et al.*, 2018; Oates *et al.*, 2020). EN devices have been widely used since the 1980s by a wide range of governmental agencies and commercial industries from biological, agricultural, forestry, food, water industry, medicine, cosmetics, *etc*. One of the most significant applications of the EN that is prevalent among various industrial uses

is quality assurance and quality control (Wilson, 2018; Al-Ramahi *et al.*, 2019; Mudalal and Abu-Khalaf, 2021).

Three commercial ENs based on MOS technology (PEN3 by Airsense Analytics, Schwering, Germany; EOS835 and EOS507C by Sacmi, Imola, Italy) have been applied successfully to distinguish infected material in a variety of pathological systems (fungal rots on kiwifruit and *Erwinia amylovora* on apple and pear plants, *Ralstonia solanacearum* or *Clavibacter michiganensis ssp.*, and *Sepedonicus* on potato tubers) (Cellini *et al.*, 2017). Researchers have demonstrated that pear trees infected with fire blight can be detected successfully in the early stages of infection using EN with MOS sensors (Cui *et al.*, 2018).

EN was also used to detect Lethal Bronzing Disease (LB) at different stages in infected cabbage palms (*Sabal palmetto*) using an array of eight MQ sensors (*i.e.* type of MOS gas sensors) (Oates *et al.*, 2020).

EN as well as other devices that rely on electronic language (*i.e.* tongue and eye) are considered in the incorporation into the input management system for the classification of complex systems like water and food samples (Fernandez-Lopez *et al.*, 2021).

2.4.3 EN advantages and limitations

EN technology has several advantages when compared to traditional methods. It is inexpensive, does not need specialists, provides high throughput detection, is rapid and has a small size. The most common types of EN obtainable on the market are metal oxide semiconductors (MOS), which have many advantages as the susceptibility to a wide range of several chemicals, rapid response and prolonged sensor life. They are also cheap, firm, and semi-selective. However, they also have a few limitations, commonly sensitive to toxicity and

moisture, high energy consumption, limited coating range and promoted sensor drift (Sanaeifar *et al.*, 2017; Abu-Khalaf, 2021).

2.4.4 EN data analysis and pattern recognition

The generated signals from EN need to be analyzed to reveal the right obtained signals. Therefore, MVDA represents a significant interpretation to reach beneficial and meaningful results. MVDA program includes a wide range of recognition and regression techniques (Abu-Khalaf *et al.*, 2004; Abu-Khalaf and Bennedsen, 2002; Abu-Khalaf and Abu Rumaila, 2020).

Numerous supervised and unsupervised techniques can be used as a recognition pattern for analyzing EN signals. However, they generally use a concerted strategy of four basic steps: the first one is calibration, which uses a set of samples for parameters optimization according to the analysis aims of the available sensor (s) and the multivariate statistical method (s) of choice. In general, an unprocessed sample before any change can be used as a reference (Park *et al.*, 2019; Rahman *et al.*, 2020). The second step is variable selection, which includes selecting the variables that contain beneficial information and removing the variables that encode noise or have no discriminatory power. Moreover, the selected variables are based on validated analytical methods, and the data set should be large enough to consider the natural variables in the problem domain (Concina *et al.*, 2009; Park *et al.*, 2019; Rahman *et al.*, 2020). The third step is to choose statistical models, by building a statistical mathematical model that is capable of describing the characteristic(s) of samples related to the selected variables, such as principal component analysis (PCA), hierarchical cluster analysis (CA), partial least squares (PLS), linear discrimination analysis (LDA), partial least squares-discriminant analysis (PLS-DA), and artificial neural networks (ANN). The fourth step is model validation and reliability by estimating

the model's recognition and prediction capabilities using an independent set of samples (Marei *et al.*, 2014; Zhang *et al.*, 2008; Abu-Khalaf and Hmidat, 2020; Najjar, 2020).

2.4.5 Principal component analysis (PCA)

PCA is considered the most popular technique for MVDA. It analyzes the statistical data in which observations are described by several variables based on nested quantities (Mishra *et al.*, 2017; Abu-Khalaf, 2019; Zaid *et al.*, 2022). The goal of PCA is to recognize the most significant basis for the re-expression of a given data set. For more explanation, PCA extracts the significant information (X matrix) from the observations and reduces the noise, then computing new linear uncorrelated variables called principal components (Kurita *et al.*, 2019; Najjar and Abu-Khalaf, 2021), which are obtained by Equation 1 (Ruiz *et al.*, 2015)

Equation 1**:** The principal components obtained equation. T: is the projected matrix to the principal component space (scores matrix), P: is the principal component of the data set (loading matrix) and E: is the residual error matrix.

$$\mathbf{X = TP^T + E}$$

Only a few numbers of PCs are kept in the analysis, where PC1 represents the maximum possible percentage of the total variance in the data set and PC2 represents the maximum residual variance, and so on. PCA can be demonstrated by a scores plot, which explains the relationships between variables, and a loading plot, which emphasize the relationships between variables (Abu-Khalaf *et al.*, 2013; Abu-Khalaf and Masoud, 2022). An example of principal component analysis (PCA) of EN data is shown in Figure 5, where the scores plot explains 98% of the total variance and different groups are identified (Park *et al.*, 2019).

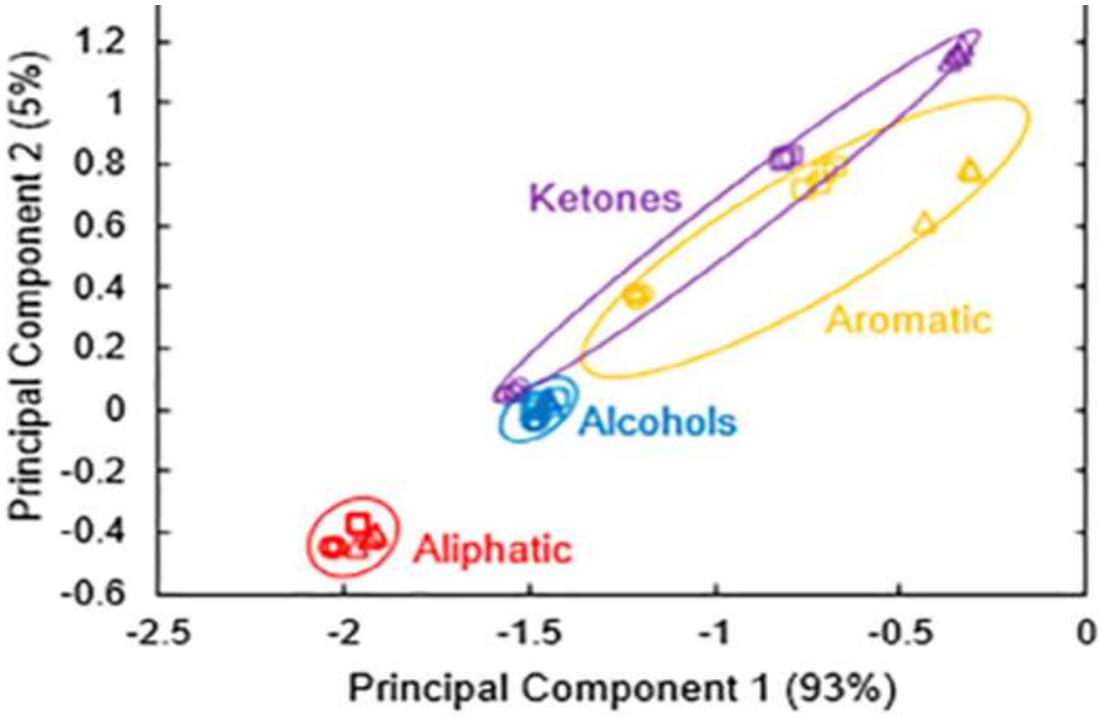


Figure 5: An example of principal component analysis (PCA) for EN data (Park *et al.*, 2019).

Chapter Three: Materials and Methods

3. Materials and Methods

3.1 Fungal method

3.1.1 Media preparation

Based on the manufacturer's instructions provided on the bottle (DIFCO Laboratories, USA) (Cat #254920), Potato Dextrose Agar (PDA) medium was prepared by dissolving 19.5 g of PDA powder in 500 ml of distilled water (DW). The solution was sterilized with an autoclaving program of 121˚C and 15 psi for one hr. After that, the purified solution was cooled enough to be poured into 9 cm sterilized Petri dishes, in which the process was done under sterilized conditions on a microbiological safety cabinet (MN 120). Finally, the media were stored in a refrigerator at 4˚C for the cultivation purposes of *C. coccodes* isolate.

3.1.2 Isolation and identification of the fungi

Samples of tomatoes infected with anthracnose were collected from Qalqilya farms (Figure 6). A small portion of the rotted area was placed on fresh PDA media and incubated at 28˚C for 72 hr under fluorescence light. After several cultivations of the growing colonies, a pure fungal isolate was obtained. The *C. coccodes* fungus was subcultured on fresh PDA media every two weeks.

Figure 6: Some tomato samples infected with *C. coccodes*.

3.1.3 Morphological identification

The main morphological features for identifying the *C. coccodes* are the shape of conidiomata and appressoria (Cano *et al.*, 2004; Liu *et al.*, 2011). Therefore, a microscopic slide of the fungus' conidiomata and appressoria was prepared and observed under the light microscope (Labomed, USA) (Cat # PN: 9135000-901). After that, the images were captured with an Aiptek HD 1080P digital camera (Aiptek International GmbH, Germany).

3.1.4 Molecular identification

3.1.4.1 Fungal DNA extraction

The DNA extraction from pure fresh colonies was applied using Promega Wizard™ Genomic DNA Purification kit (Cat# A1120), where about 100 mg of

pure fungus' isolate was added to 1 ml of autoclaved DW in a 1.5 ml microcentrifuge tube, then centrifuged for 2 min at 10000 rpm. After discarding the resulted supernatant, 600 µl of nuclei lysis buffer was added to the cell pellet and gently mixed. later, the microtube was vortexed vigorously at high speed for 20 sec. The sample was incubated at 65˚C for 15 min, and 3 µl of RNAs was added, then vortexed again. Afterward, the sample was at 37˚C for 15 min, left to rest for 5 min at room temperature, and 200 µl of protein precipitation solution was added to the tube with vigorous vortexing. The tube was centrifuged for 3 min at 10000 rpm, supernatant (with DNA) was transferred to a clean 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol, and gently mixed to be centrifuged at 10000 rpm for 1 min. The next step was to decant the supernatant carefully and drying on a clean absorbent paper. Then, 600 µl of room temperature 70% ethanol was added and gently mixed to wash the DNA pellet, centrifuged at 10000 rpm for 1 min, the ethanol was carefully aspirated, and the tube was drained and air-dried for 15 min. After that, 100 µl of DNA rehydration solution was added to the tube. In the end, the DNA was rehydrated by incubating the solution overnight at room temperature or 4˚C. After checking the DNA by gel electrophoresis, it was kept at -20˚C for further use (Han *et al.*, 2015).

3.1.4.2 Polymerase chain reaction (PCR)

Three different primer sets were used for the identification of *C. coccodes* (Table 1). The first primer set was ITS1F/ITS4, which was used to amplify the DNA of most *Ascomycetes* and *Basidiomycetes* (Belov *et al.*, 2018). The other two primer sets are Cc1F1/Cc2R1 and Cc1NF1/Cc2NR1. Where, Cc1F1/Cc2R1 was a genus-specific outer primer set that is specific to *Colletotrichum* spp., amplified a single product of 447 bp. Cc1NF1/Cc2NR1 was a species-specific

nested primer set that is unique to *C. coccodes*, amplified a single product of 349 bp (Cullen *et al.*, 2002).

First of all, DNase-free water was added to primers to obtain a final 100 µM concentration according to their molecular weight. For each primer set, the PCR was achieved in a 25 µl reaction volume. Each reaction mixture contained 11µl of 2X GoTaqVR Green Master Mix (Promega Corporation, USA) (Cat # M7128), 1.5 µl of $MgCl_2$, 0.2 µl of each primer in each set, 1 µl of the DNA and 11.1 µl of ultra-pure DNase free water (Biological Industries, Cat # P119A, USA). PCR reagents with 1 µl dH_2O (instead of DNA) were used as negative controls. For nested PCR, 1 µl of the first-round PCR product was used. The PCR products of the ITS1F/ITS4 primer set were sent for sequencing at Bethlehem University/Palestine. The sequence identification was carried out by BLASTn Analysis Database (NCBI).

3.1.4.3 The PCR amplification program

The amplification program with ITS1F/ITS4 set was carried out using Verti^{TM} 96 well thermal cycler: Cat # 4375786 (Applied Biosystems Company, California, USA), with the following PCR program: initial denaturation cycle for 3 min at 94˚C, then 30 cycles for 30 sec at 94˚C, annealing for 30 sec at 54˚C, followed with elongation for 45 sec at 72˚C and the final cycle for 5 min at 72˚C.

For nested PCR a two-step amplification program was performed using Verti^{TM} 96 well thermal cycler: Cat # 4375786 (Applied Biosystems Company, California, USA), in which the single-round PCR with Cc1F1/Cc2R1 primer set included an initial denaturation cycle at 95˚C for 2 min, 35 cycles for 45 sec at 95˚C, annealing for 1 min at 61˚C followed by extension for 90 sec at 72˚C and a final cycle for 5 min at 72˚C. The second-round PCR with Cc1NF1/Cc2NR1

primer set, involved an initial denaturation cycle at 95˚C for 2 min, 35 cycles for 45 sec at 95˚C, annealing for 45 sec at 72˚C followed by extension for 90 sec at 72˚C and the final cycle for 5 min at 72˚C.

Table 1: List of primer sets used for *C. coccodes* identification.

Primer	Sequences (5˙-3˙)	Band size (bp)	Annealing temperature (˚C)	Reference
ITS1F	CTTGGTCATTTAGAGGAAGTAA	400	54	Belov *et al.*, 2018
ITS4	TCCTCCGCTTATTGATATGC			
Cc1F1	ACCTAACTGTTGCTTCGGCG	447	61	Cullen *et al.*, 2002
Cc2R1	AAATTTGGGGGTTTTACGGC			
Cc1NF1	TGCCGCCTGCGGACCCCCCT	349	72	Cullen *et al.*, 2002
Cc2NR1	GGCTCCGAGAGGGTCCGCCA			

3.1.4.4 Gel electrophoresis for extracted DNA

Agarose gel of 1% concentration was prepared to run the extracted DNA, which was prepared by dissolving 1 g agarose powder in 100 ml of 0.5 X Tris-borate-ethylenediaminetetraacetic acid (EDTA-TBE) buffer with heating, then cooled at room temperature. A 1.5 µL of 10000x gel red DNA stain (Cat # 41003) was added and poured into a tray (20*20 cm) of the Submarine Horizontal type electrophoresis system. After that, 5 µl of the DNA with 2 µl of blue orange loading dye 6x (Cat # 25223004) were mixed and loaded into the well of the gel, in addition to 3 µl of 1 kilobase-pairs (kbp) plus ladder (Cat # DM001-R500). After running the device for 30 min at 70 volts, the results were visualized and

recorded using an ultraviolet (UV) illuminator device (Uvitec, Cat # 1210234, Cambridge) and a gel documentation system.

3.1.4.5 Gel electrophoresis for PCR products

Agarose gel of 1.5% concentration was prepared by dissolving 1.5 g of agarose powder in 100 ml of TBE buffer with heat using the microwave. Followed by the addition of 1.5 µl of 1000x gel red and pouring of the mix into 20*20 cm of the Submarine Horizontal type tray. After that, 7 µl of PCR product was loaded, in addition to 3 µl of 1 kilobase- pairs (kbp) plus ladder (Cat # DM001-R500). After running the device for 1 h, the resulted bands were viewed and recorded using a UV illuminator device (Uvitec, Cat # 1210234, Cambridge) and a gel documentation system.

3.2 Prepare the *C.coccodes* suspension and tomato infection

The conidial suspension was prepared from seven days-old cultures of *C. Coccodes* according to Rodriguez-Salamanca *et al.* (2018), in which the culture was immersed with 1.0 ml of sterile double-distilled water (DDW) containing 0.001% tween 80. Conidia were harvested by gently scratching the surface with a sterile glass rod. Three layers of sterile cheesecloth were used for conidial suspension filtration. Then the conidial concentration was adjusted to $30*10^3$/ml concentration under the optical microscope using a hemocytometer. Second and third adjustments were made to attain $30*10^4$ and $30*10^5$ conidia/ml concentrations. The conidial suspensions were kept to be used for inoculating tomato samples.

3.3 Tomato samples sterilization and injection

Sterilization and injection of tomato samples were applied as described by Jones *et al.* (2002) and Ben-Daniel *et al.* (2009). The collected tomato fruits were

surface sterilized with 3% sodium hypochlorite (NaOCl) for 10 min, washed completely with sterile DW, and left in a laminar hood to dry at room temperature. Then, the tomato samples (each sample had a replicate) were inoculated with the conidial suspension by wounding to a depth of 2-3 mm and injecting 10 µl of the suspension at four points around the longitudinal axis using a sterile needle. Samples injected with sterile DW were used as controls.

3.4 Storage condition

Samples were examined every 2 days until the 10^{th} day using the EN device. The samples were labeled and stored in an incubator for 10 days under continued fluorescent light and 28˚C temperature (Figure 7) (Hong and Hwang, 1998; Aqeel *et al.*, 2008).

Figure 7: Tomato samples stored in an incubator; under fluorescent light and at 28˚C.

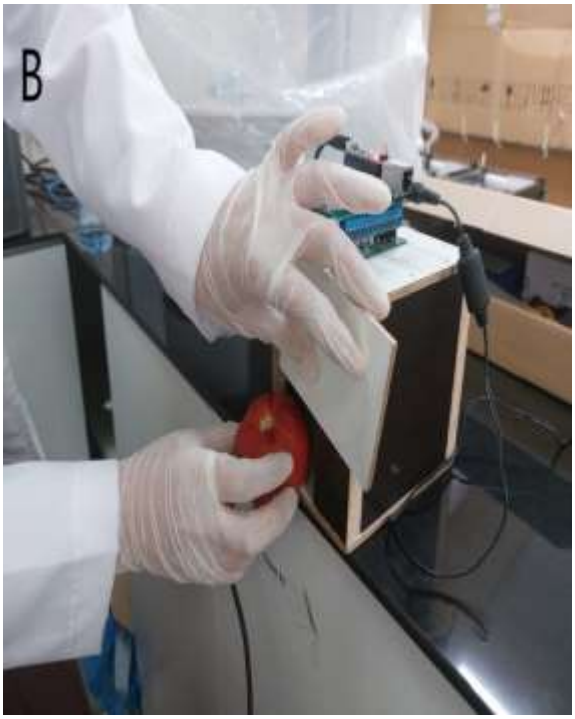
3.5 EN measurement

A prototype EN with eight metal-oxide semiconductors (MOSs) (Hanwei Electronics Co., Ltd., Zhengzhou, China) was used for the measurement of tomato samples (Figure 8). The specification of the MOS is found in Table 2 (Abu-

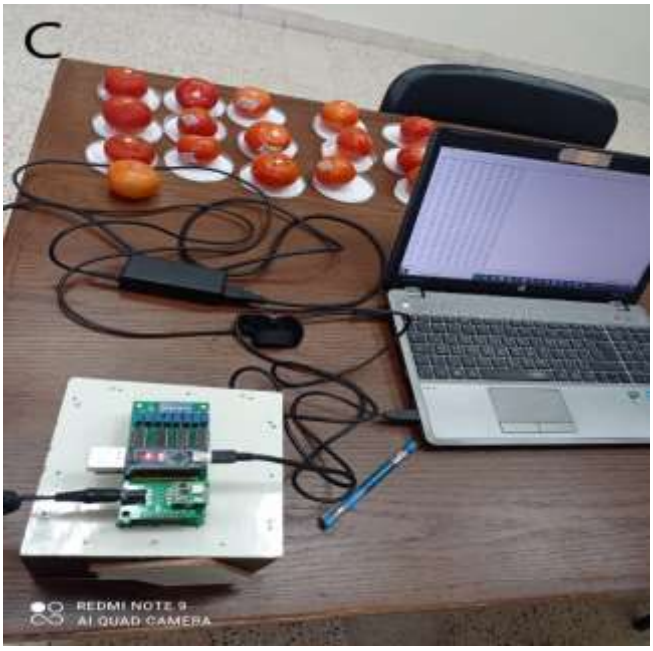
Khalaf, 2021). Each tomato sample was inserted inside a homemade box for about 3 min and then tested in triplicates. The steps for measuring samples by EN are shown in Figure 9.

Figure 8: A prototype electronic nose (EN) with eight metal-oxide semiconductors (MOS) sensors.

Samples were kept under controlled conditions in an incubator at 28˚C



The samples were put in wooden box for measurement



Each sample was tested for 3 min by EN device

Raw data obtained for further processing by MVDA

Figure 9: Steps for measuring samples (A-D) using electronic nose (EN).

Table 2: Metal-oxide sensors (MOSs) specifications used in electronic nose (Abu-Khalaf, 2021).

Sensor Name	Target Gas Sensitivity	Typical Detection Ranges (ppm)
MQ-2	General combustible gas	200–5000 liquefied petroleum gas (LPG) and propane, 300–5000 butane, 5000–20,000 methane, 300–5000 hydrogen (H_2), 100–2000 Alcohol.
MQ-3	Alcohol vapor	10–300.
MQ-4	Natural gas and methane	200–10,000 CH_4, natural gas.
MQ-5	LPG, natural gas, and coal gas	200–10,000 LPG, liquefied natural gas (LNG), natural gas, iso-butane, propane, and town gas.
MQ-6	LPG, propane	200–10,000 LPG, iso-butane, propane, LNG.
MQ-8	Hydrogen	100–10,000.
MQ-135	Air quality control (NH_3, benzene, alcohol, smoke)	10–10,000.
MQ-138	Formaldehyde, benzene, aldehyde, ketone and ester	10–1000 benzene, 1–1000 alcohol, 10–3000 NH_3.

3.6 EN data analysis

Unscrambler (version 10.3, Camo Software AS, Oslo, Norway), a multivariate data analysis (MVDA) program, was used for EN data analysis.

3.7 PCA analysis

To study the ability of the EN device to differentiate between infected samples and control samples at the different stages of *C.coccodes* fungal growth, PCA models for the measured EN signal values were implemented.

Chapter Four: Results and Discussion

4. Results and Discussion

4.1 Identification of *C.coccodes* fungus

4.1.1 Morphological identification

The results obtained from the morphological identification of *C.coccodes* are shown in

Figure 10. The conidia were straight; fusiform; with acute ends and are often slightly constricted in the middle. The appressoria (thick-walled swellings at the end of a hypha) were abundant, ellipsoidal, irregularly lobed or irregularly curved, borne on vitreous supporting filaments, thin walled supporting hyphae, with a changing shape (Figure 10).

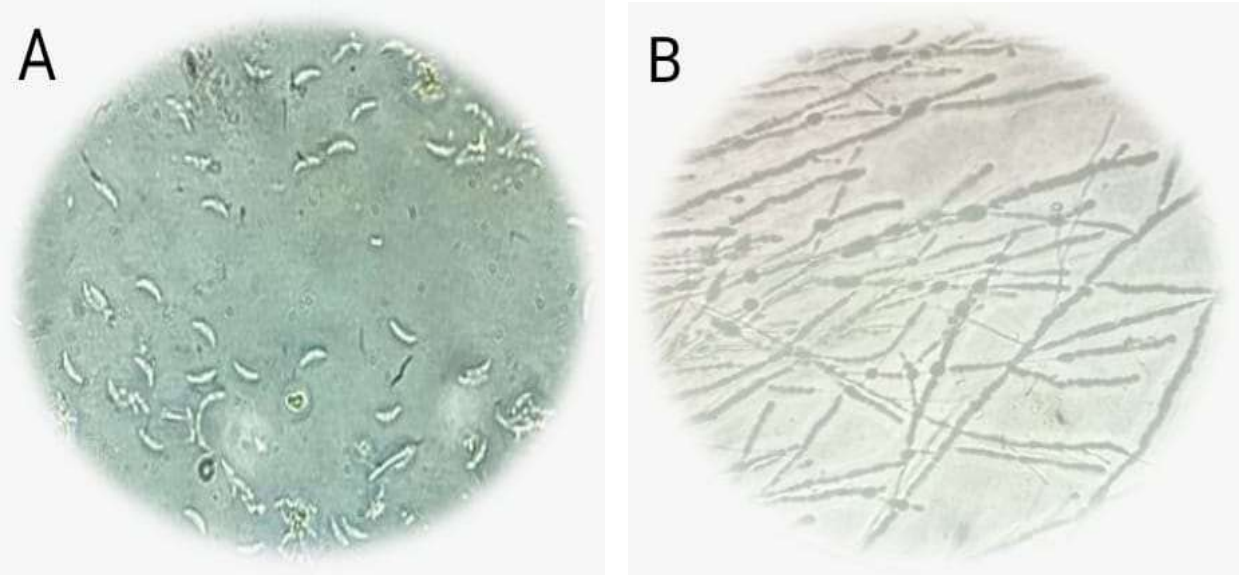


Figure 10: Morphological characteristics of *C. coccodes*. A: The conidia shape under the light microscope with 40X. B: The appressoria shape under the light microscope with 40X.

Colonies on PDA are circular, initially white and becoming gray with age, forming black spots on agar, have white, cottony mycelia without visible conidia

masses, weak sporulation and white to pale yellow inverted (Figure 11). These results are similar to many studies that identified *C. coccodes* previously according to the shape of conidiomata and appressoria (Cano *et al.*, 2004; Cannon *et al.*, 2007; Liu *et al.*, 2011).

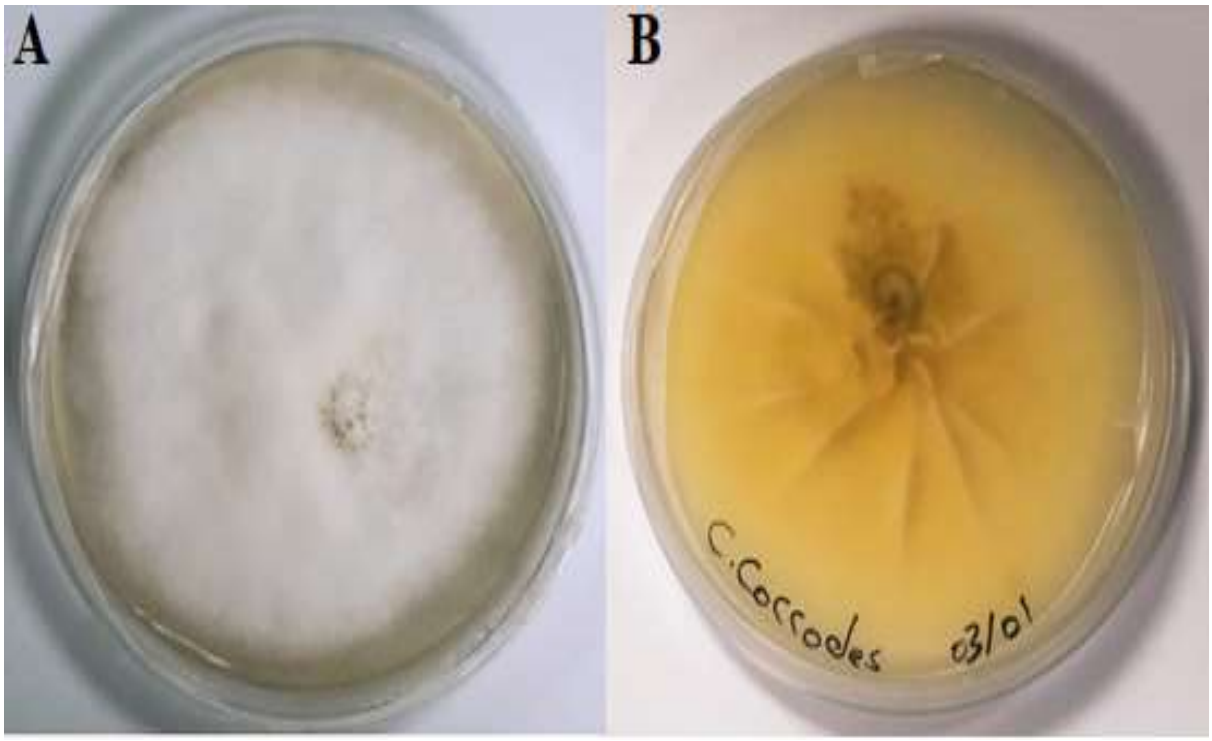


Figure 11: Pure *C.coccodes* fungus on PDA media. A: The front view. B: The reverse view.

4.1.2 Molecular identification using a PCR

4.1.2.1 Total DNA extraction

The total DNA extracted from the sample cultures is shown in Figure 12 using the Promega Wizard™ Genomic DNA Purification Kit. The results exhibited the existence of an amount of DNA in the isolated samples.

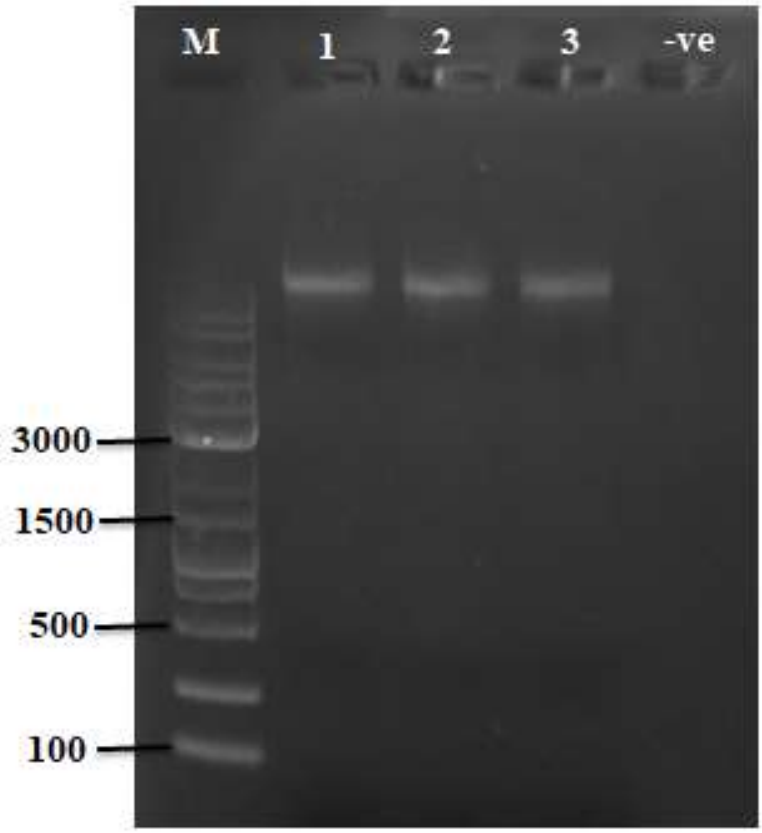


Figure 12: Gel electrophoresis documented photos of total DNA isolated from three suspected *C. coccodes* fungal isolates. Where lanes from 1-3 represent replicate of the isolated fungi, -ve is a negative control. M is a 1 Kbp plus ladder as a molecular size marker.

4.1.2.2 Primers used in *C. coccodes* detection

The results from the three different primer sets (ITS1F/ITS4, Cc1F1/Cc2R1 and Cc1NF1/Cc2NR1) indicated high specificity in *C. coccodes* detection. The result with the primer set ITS1F/ITS4 indicated the presence of either *Ascomycetes* or *Basidiomycetes* isolates among the three tested samples, where isolates one and two showed a positive result for either of these fungal types with a band of approximately 400 bp (Figure 13) as confirmed by Ayad *et al.* (2017).

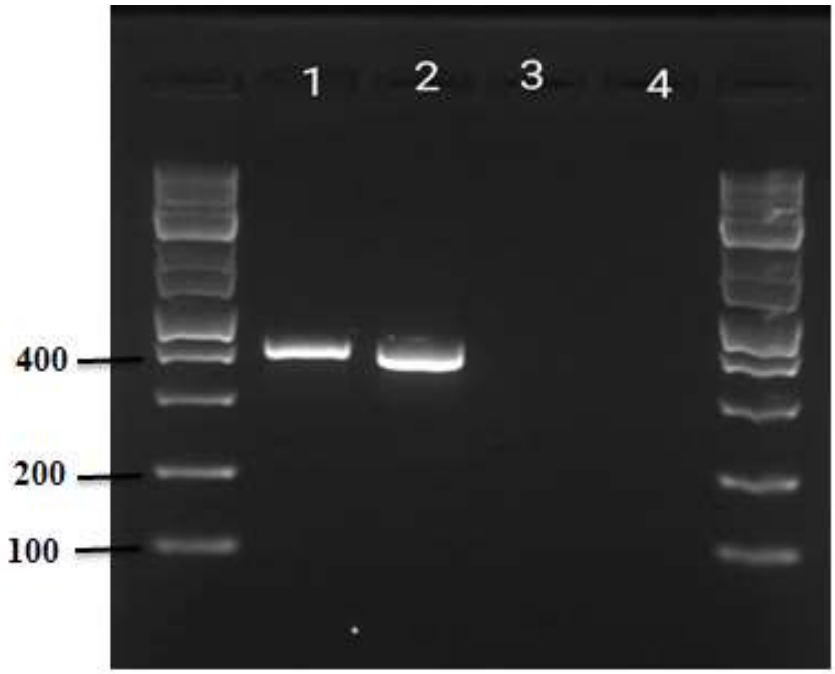


Figure 13: Gel electrophoresis documented photos of the PCR product used for the ITS1F/ITS4 primer set. Where lanes from lanes 1 and 2 are a positive result for *Askomycetes,* lane 3 is a negative result and lane 4 is a negative control. M is a 100 bp ladder as a molecular size marker.

The primer set Cc1F1/Cc2R1 is specific to *Colletotrichum* spp., which resulted in aband of the 447 bp fragment. The result in Figure 14 indicates that the two isolated fungal products belong to *Colletotrichum* spp. In addition, the result of primer set Cc1NF1/Cc2NR1, which is unique to *C. coccodes*, generated

a 345 bp fragment indicating a positive result for *C. coccodes* (Figure 14) (Cullen *et al.*, 2002). The sequence identification was carried out by BLASTn Analysis Database (NCBI), where the similarity for the *C. coccode* isolate sequence identification was 89% as shown in Figure 15. The percentage may be low due to contamination from the isolation method, but the genus-specific primers that were used solved this problem. These results are consistent with several previous studies (Cullen *et al.*, 2002; Martinez-Culebras *et al.*, 2003; Ayad *et al.*, 2017; Belov *et al.*, 2018).

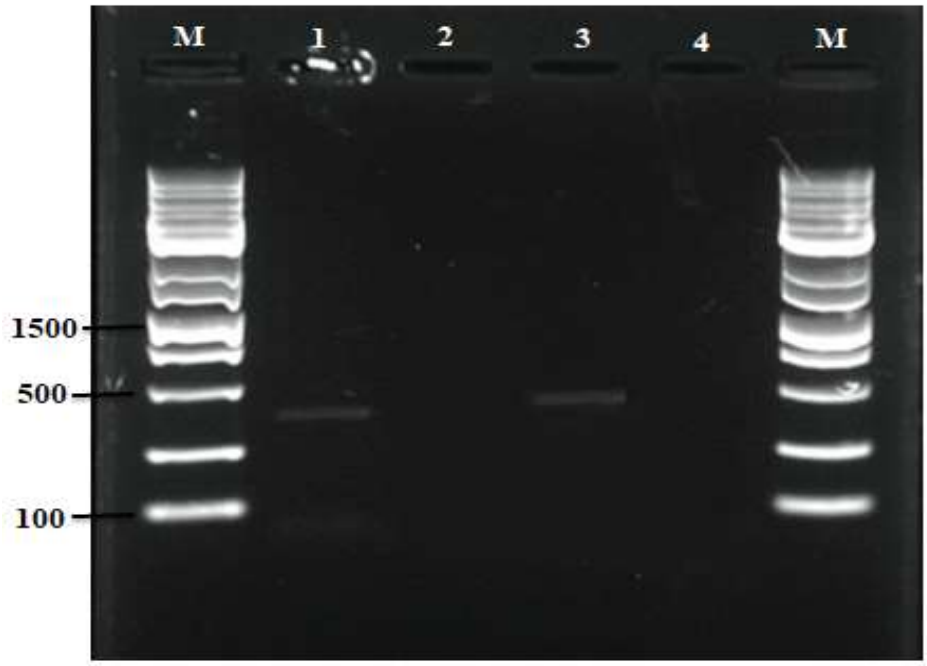


Figure 14: Gel electrophoresis documented photos for the PCR products using the two specific primer sets (Cc1F1/Cc2R1, Cc1NF1/Cc2NR1). Where lane 1 is a band with Cc1NF1/Cc2NR1 primer set, lane 2 is a negative control for Cc1NF1/Cc2NR1, lane 3 is a band with Cc1F1/Cc2R1 specific primer set, and lane 4 is a negative control for Cc1F1/Cc2R1. M is a 1 Kbp ladder as a molecular size marker.

Download GenBank Graphics ▼Next ▲Previous ◀◀Descriptions

Colletotrichum coccodes 5.8S rRNA gene, internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), strain IMI 363581

Sequence ID: AJ536227.1 **Length:** 482 **Number of Matches:** 1

Range 1: 172 to 345 GenBank Graphics ▼Next Match ▲Previous Match

Score	Expect	Identities	Gaps	Strand
225 bits(249)	5e-60	155/174(89%)	1/174(0%)	Plus/Plus

```
Query  288  TACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCG  347
            ||  ||||||||||||||||||||||| ||| |||||||||||||||||||||||||||
Sbjct  172  TAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG  231

Query  348  ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCT  407
            ||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  232  ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCT  291

Query  408  CCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA-TTAAATTCTCAACCTC  460
            |    | |||| |  ||||||||||||| ||| |||| || ||  ||||| |||
Sbjct  292  CGCCAGCATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTC  345
```

Figure 15: BLASTn similarity for the *C.coccode* isolate sequence identification.

4.1.3 PCA analysis

PCA scores plot of the second day of injection with *C. coccode* fungus is shown in Figure 16. Two principal components (PCs) explained 100% of the total variance. The first principal component (PC-1) explained 98% of the total variance and the second PC-2 explained 2%. While on the fourth day of injection, the PC-1 explained 97% and the PC-2 explained 3% of the total variance, but the separation distance between the control and the sample values in the PCA scores plot of the fourth day did not change much compared to the second day as shown in Figure 17. In these two PCAs, the results showed the ability of EN to differentiate between the infected sample and the control. Although the symptoms were not obvious to the naked eye at this point.

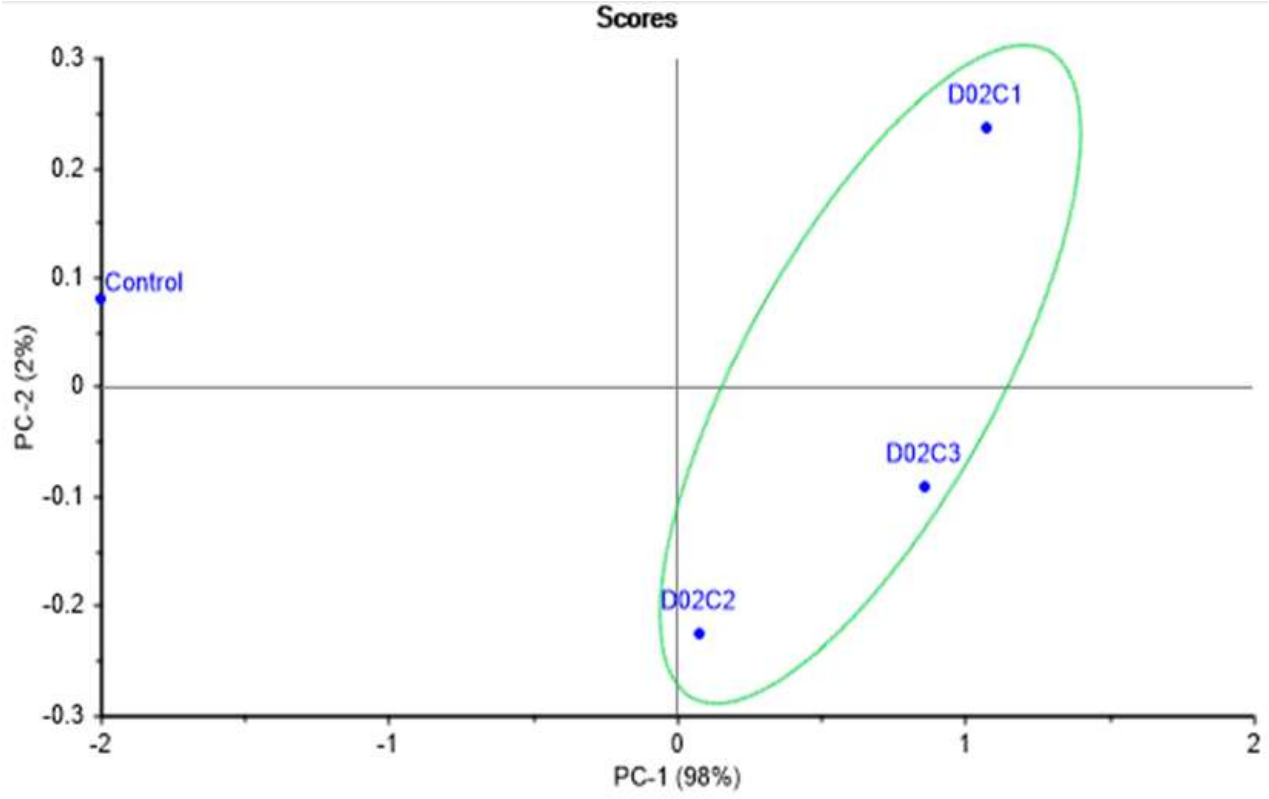


Figure 16: PCA scores plot for measured infected tomato samples with *C.coccodes* on the second day of infection using electronic nose (EN). The two PCs explained 100% of the total variance.

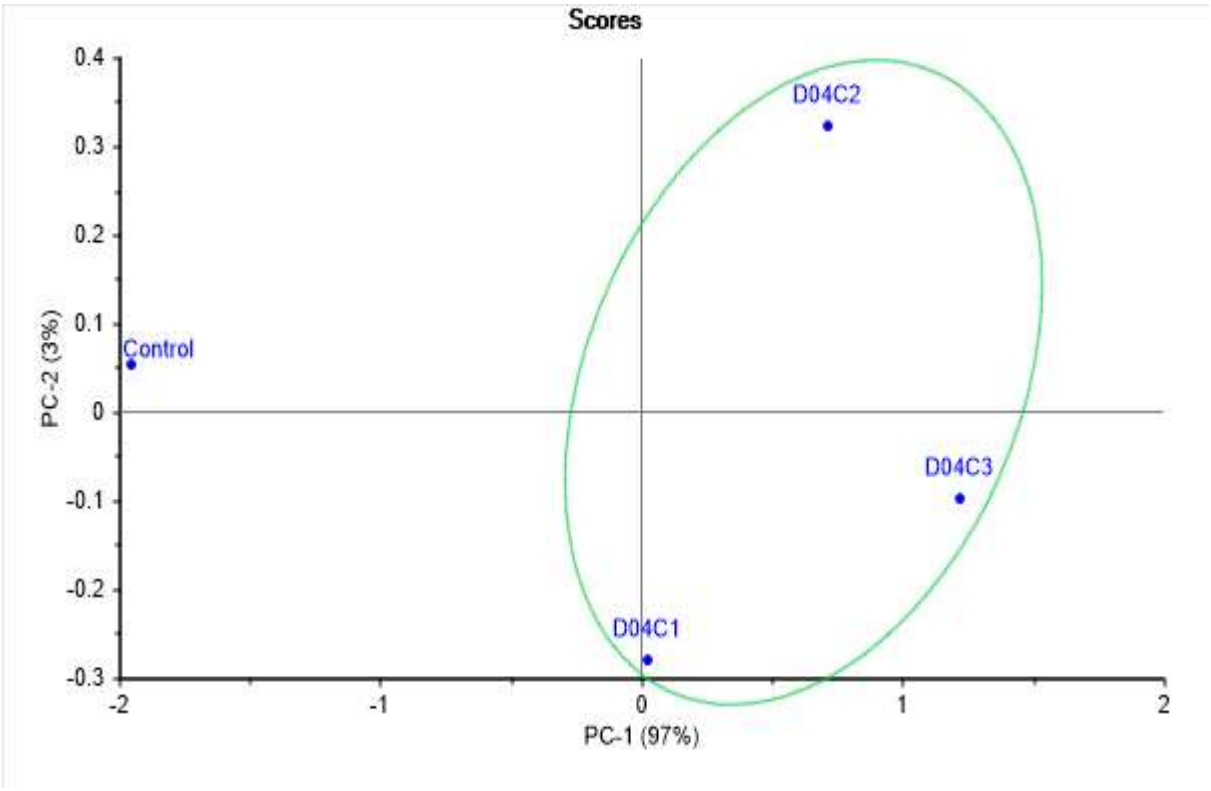


Figure 17: PCA scores plot for measured infected tomato samples with *C.coccodes* on the fourth day of infection using electronic nose (EN). The two PCs explained 100% of the total variance.

On the sixth day of injection, the PCA scores plot (Figure 18) showed that the PC-1 explained 99% and the PC-2 explained 1% of the total variance. This provides 100% of the total variance. The result of this PCA showed that EN discriminates between the control and the infected samples. Where at this point (*i.e.* 6 day) the symptoms start to become visible after inoculation (Tsitsigiannis *et al.*, 2008).

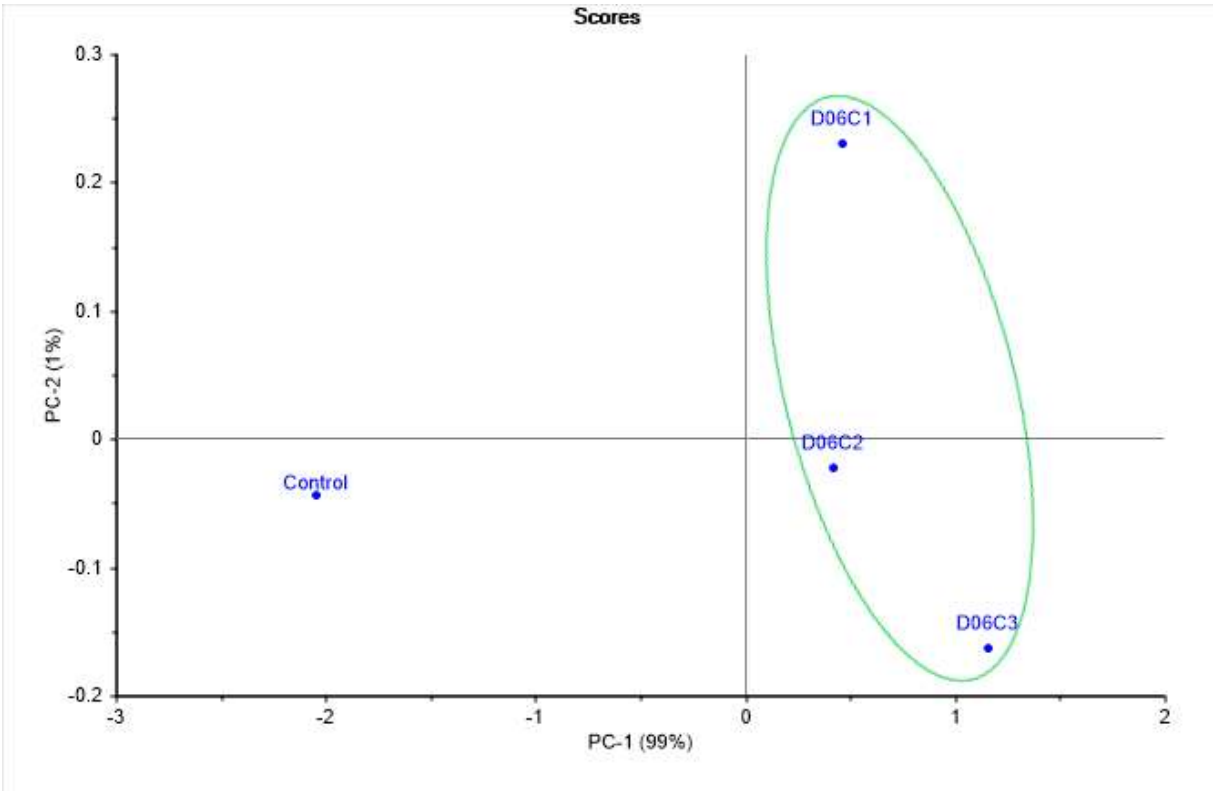


Figure 18: PCA scores plot for measured infected tomato samples with *C.coccodes* on the sixth day of infection using electronic nose (EN). The two PCs explained 100% of the total variance.

On both the eighth and tenth days the PC-1 explained 100% of the total variation. This indicates that the two PCAs were able to fully classify between infected and control samples, as shown in Figure 19 and Figure 20.

Figure 21 shows the overall PCA scores plot for measured infected tomato samples with *C. coccodes* using electronic nose (EN). The two PCs explained 100% of the total variance. The separation distance between the values of the control and the infected samples began to increase. While the distance between the values on the second and fourth days has some overlapping. The concentration of infected samples can be clearly differentiated on the eighth and tenth days. These results indicate that the EN has the potential to differentiate between the different stages of tomato infected with *C.coccodes*.

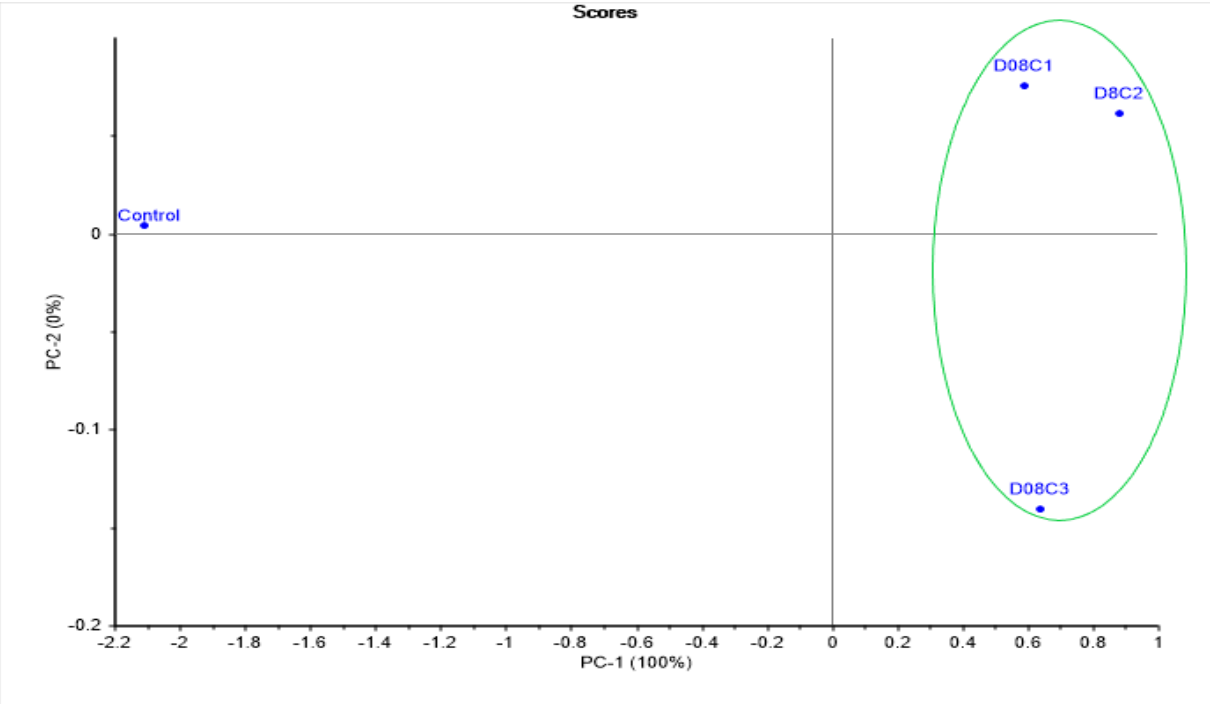


Figure 19: PCA scores plot for measured infected tomato samples with *C.coccodes* on the eighth day of infection using electronic nose (EN). PC-1 explained 100% of the total variance.

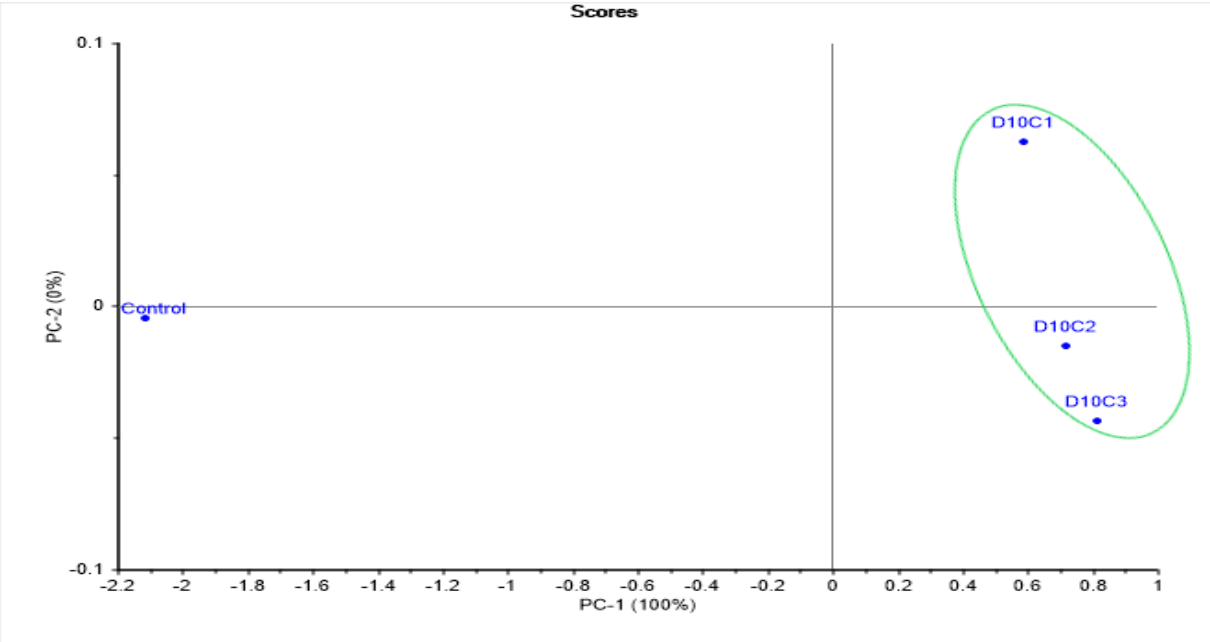


Figure 20: PCA scores plot for measured infected tomato samples with *C.coccodes* on the tenth day of infection using electronic nose (EN). PC-1 explained 100% of the total variance.

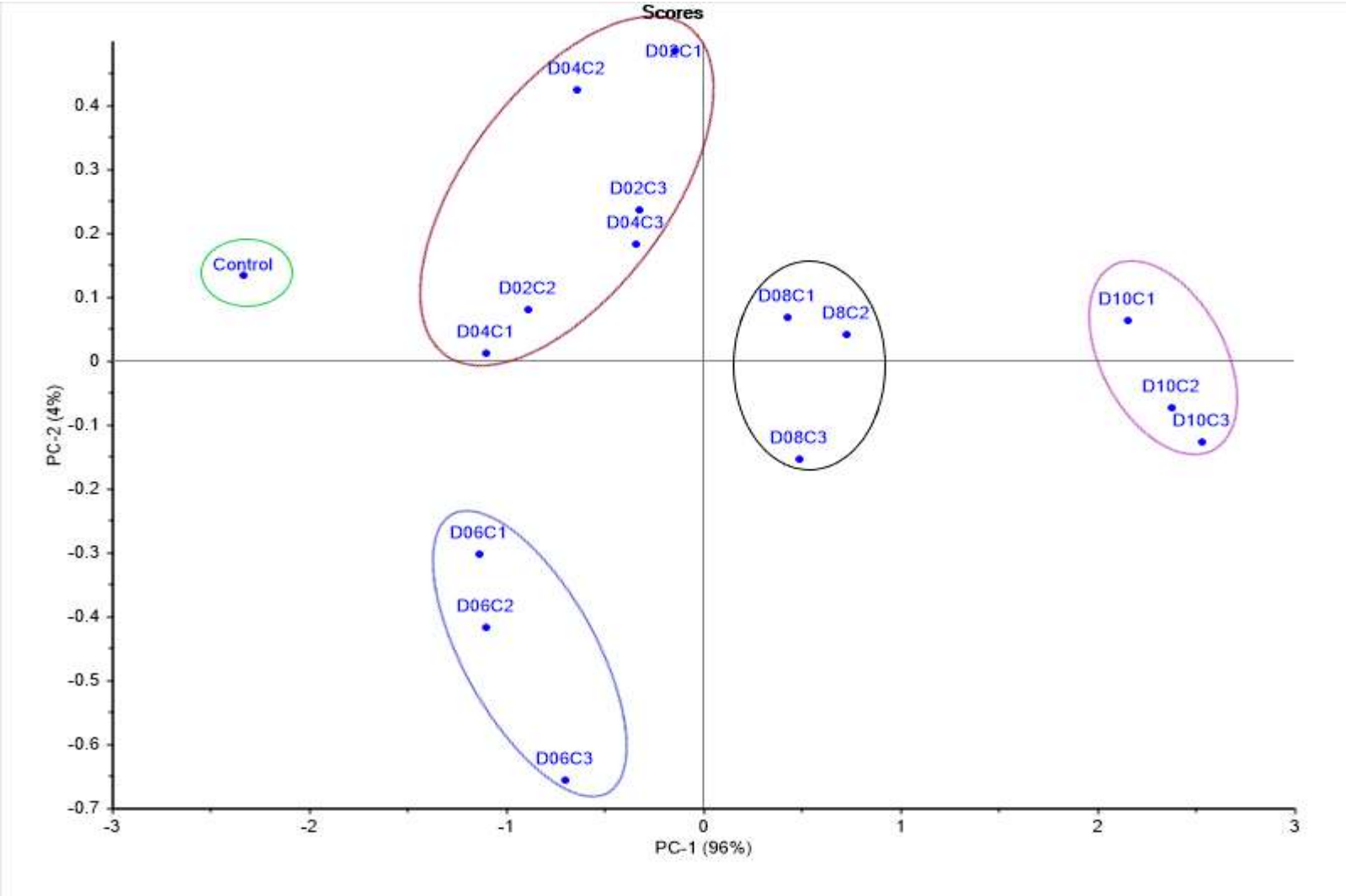


Figure 21: The overall PCA scores plot for measured infected tomato samples with *C. coccodes* using electronic nose (EN). The two PCs explained 100% of the total variance. Where the values of the second and fourth days have some overlapping, and the other values on the sixth, eighth and tenth days are completely separated.

The factor responsible for anthracnose symptoms caused by C. *coccodes* is triggered by ammonia secretion and accumulation along with other VOCs during the infection period (Palaniyandi *et al.*, 2013). This explains the ability of the EN with its MOS sensors to sense the presence of the *C. coccodes* on the second day of infection and to distinguish between the different stages of infection. These PCA results are in agreement with several previous studies (Ghaffari *et al.*, 2010; Zhang *et al.*, 2011; Cheng *et al.*, 2017; Verma *et al.*, 2018; Oates *et al.*, 2020). For example, Zhang *et al.* (2011) used EN to estimate the chemical and physical properties of pear. In addition, EN was used in the prediction of the presence of the food spoilage yeast *Debaryomyces hansenii* by MVDA (Masoud *et al*., 2021).

Another study used EN based on MOS in the detection of the type and concentration of VOCs to determine the stage of crop diseases and insect pests (Zheng and Zhang, 2022). Biondi *et al.* (2014) used EN to detect potatoes infected with *Ralstonia solanacearum* which causes potato brown rot and used PCA to analyze samples.

Wheat from five storage ages with 15 degrees of insect damage was assessed and classified using PEN2-EN, which consists of 10 MOS sensors that were used to create a typical chemical fingerprint of VOCs present in the samples. Wheat samples were classified into five groups of wheat of different storage ages and 15 groups of different grades of insect-damaged wheat using PCA and LDA. These results indicated the ability of EN to distinguish between wheat of different ages and different degrees of insect damage (Zhang and Wang, 2007).

EN with MOS and its rGO-catalyzed compounds (Pd, Pt, Au) with nanorods/nanowires morphology are found to have excellent sensing properties

of NO2 gas, which is used in monitoring environmental NO2 and its effect on plant ecosystem (Tyagi *et al*., 2022).

EN was used as a rapid method for early detection and classification of fungal infection on garlic that has been synthetically infected with *Fusariumoxysporum f. sp. Cepae* (FU), *Alternariaembellisia* (syn. *Embellisiaallii*) (AL), and *Botrytis allii* (BO). Where the aroma profile obtained by EN was analyzed using PCA, LDA and other MVDA techniques (Makarichian *et al*., 2022).

A conductive polymer gas sensor array was used to detect and classify blueberries infected with gray mold (*Botrytis cinerea*), anthracnose (*Collecotrichum gloeosporioides)* and Alternaria mold (*Alternaria spp*.), which are common post-harvest fungal pathogens. The data analysis was achieved using PCA. This study emphasize the potential feasibility of using a gas sensor array to assess post-harvest blueberry quality and detect fungal diseases (Li *et al*., 2009).

Chapter Five: Conclusion and Recommendations

5. Conclusion and Recommendations

5.1 Conclusion

In conclusion, this study highlighted the efficiency of the portable EN device in identifying the *C. coccode* fungus and distinguishing between its different stages since the beginning of the disease. Therefore, it could be used as an alarm system in the field and in food storage. Several conclusions can be drawn from previous work:

1. Specific primers (*i.e.* ITS1F/ITS4, Cc1F1/Cc2R1, and Cc1NF1/Cc2NR1) could recognize *C. coccode* fungus using the PCR technology and sequencing.

2. The EN device was able to sense the *C. coccode* fungus in early-stage on tomato fruits, *i.e.* the second day.

3. The EN device was able to differentiate between the different stages of *C. coccodes* in tomatoes using EN.

5.2 Recommendations

1. In perspective, EN analysis on plant fruits could be used to aid other diagnostic techniques, such as PCR-based techniques, to provide a fast examination of samples and improve time and resource adherence in pathogens detection.

2. EN sensors could be used to aid in quality control of plant disease and detecting insect pests.

3. Improved data processing and analysis capabilities will increase the chance of providing diagnosis with higher efficiency and greater accuracy in the EN system for the detection of plant pathogens.

4. To enhance the performance of the sensor, the selectivity and sensitivity of the sensors can be improved by using nanostructured surfaces and a metal-organic framework, which can reduce the sensitivity of the sensor to external influences. In addition to using other sensors in the EN device and replacing some of the existing ones.

Chapter Six: الملخص

الملخص

الكشف عن فطر *Colletotrichum coccodes* المسبب لعفن الفاكهة (anthracnose) في البندورة باستخدام الأنف الإلكتروني

يعد الفطر (*Colletotrichum coccodes*) من أكثر عزلات *Colletotrichum* التي يصعب السيطرة عليها. بمجرد إصابة البندورة بالفطر المذكور، يتطور مرض عفن الفاكهة (*anthracnose*) في غضون أيام قليلة. لذلك فإن الكشف المبكر عن العدوى الفطرية أمر حيوي للوقاية الناجحة والعلاج الفعال للحقول المصابة. هدفت هذه الدراسة إلى استخدام الأنف الإلكتروني (EN) مع مستشعرات أكسيد المعادن (MOS) لاستشعار وجود فطر *C. coccodes* على ثمار البندورة في مراحل وتركيزات مختلفة.

حيث بدأت الدراسة بتحديد هوية *C. coccodes* باستخدام تفاعل البوليميراز المتسلسل (PCR)، وتم التعرف على فطر*C. coccode* باستخدام ثلاثة برايمارات مختلفة ITS1F / ITS4 و Cc1F1 / Cc2R1 و Cc1NF1 / Cc2NR1. حيث كان ITS1F / ITS4 عبارة عن محدد عام لمعظم فطريات *Ascomycetes* و *Basidiomycetes*، كانت المجموعة Cc1F1 / Cc2R1 عبارة عن محدد أساسي لفطر .*Colletotrichum* spp، والذي يعطي ناتج بحجم تقريبي 447 قاعدة نيتروجينية، وكان Cc1NF1 / Cc2NR1 محددا أساسًيا وفريدًا لفطر *C. coccodes*. مما ينتج عنه ناتج بحجم تقريبي 349 قاعدة نيتروجينية. بالإضافة إلى ذلك، تم حقن ثلاثة عينات بندورة متجانسة

بثلاثة تراكيز مختلفة (30 * 10^3، 30 * 10^4، 30 * 10^5) من المعلق الفطري، وثلاثة عينات بندورة أخر سليمة استخدمت لمعايرة الجهاز، وتم قياسها جميعًا باستخدام جهاز EN كل يومين لمدة عشرة أيام.

تم تحليل البيانات التي تم الحصول عليها من مستشعرات EN باستخدام تحليل المكون الرئيسي (PCA). حيث وجد أن جهاز EN يمكن أن يستشعر حدوث مسببات الأمراض في المراحل المبكرة، أي بعد يومين من الإصابة، وقبل ظهور أي أعراض، حيث أوضح تحليل المكون الرئيسي 100٪ من التباين الكلي. بالإضافة إلى ذلك، أوضح تحليل المكون الرئيسي الإجمالي 100٪ من التباين الكلي الذي يشير إلى قدرة EN على التفريق بين المراحل المختلفة من مدة الإصابة (أي اليوم الثاني والرابع والسادس والثامن والعاشر). علاوة على ذلك، تباين تركيز العينات المصابة بشكل واضح في اليوم السادس والثامن والعاشر.

Chapter Seven: References

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