AN ON-SITE BIOSENSOR-IoT SYSTEM FOR EARLY DETECTION OF RICE BACTERIAL LEAF BLIGHT IN MUDA AGRICULTURAL DEVELOPMENT AUTHORITY (MADA) SMART FARMING FIELDS

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ABSTRACT

The biosensor system designed for the early detection of rice bacterial leaf blight (BLB) has been effectively implemented at the Muda Agricultural Development Authority (MADA) Smart Farming Field located in Kampung Bohor, Alor Setar, Kedah. This comprehensive detection system consists of antibody-functionalized screen-printed carbon electrodes (SPCEs), a portable electrochemical reader linked to an Android device with the MARDIsense app, and a cloud computing-enabled web server. A total of 10 sampling points from four (4) rice plots (namely, Plot A, B, C1, and C2) were evaluated for BLB disease determination at 32 days after sowing (32 DAS) on 13 December 2023. A total of 28 samples were collected, with three replicates obtained for each sampling point, except for Point 6 in Plot B, which had only one replicate due to the rice plant's growth stage at 20 DAS during the sampling period. Simple sample preparation methods were employed, and analysis was conducted in situ. During analysis, a droplet of the extracted sample was applied to the modified SPCEs connected to the portable electrochemical biosensor device. The obtained results were quantitatively measured and categorized as 'Not Detected,' 'Low,' 'Medium,' and 'High,' indicating different levels of disease incidence based on preset thresholds for bacterial concentrations. Notably, out of the 28 samples, 26 showed no BLB disease incidence (92.86%), while only two samples displayed a low BLB incidence with bacterial concentrations below 102 CFU/mL. The analyzed data were securely stored on a cloud server accessible via a dedicated web server. The field results were cross-validated using enzyme-linked immunosorbent assays, demonstrating an excellent correlation and affirming the accuracy of the biosensor system deployed on-site.

Keywords: bacterial leaf blight, biosensor, early detection, MARDIsense app, real time monitoring, rice disease

INTRODUCTION

Rice holds significant importance within Malaysia's agricultural landscape, covering approximately 647,859 hectares of land. Serving as a vital commodity for the nation's food production, rice stands as a staple in the diets of Malaysians. The Muda Agricultural Development Authority (MADA), known as MADA, is a prominent extension agency managing the largest expanse of rice fields, spanning 100,785 hectares in Kedah and Perlis in northern Malaysia (Chompa et al. 2022). However, the nation's rice production consistently faces challenges due to recurring attacks by various rice diseases, resulting in significant yield and economic losses for farmers and the agricultural sector. Rice diseases can occur at various stages of rice cultivation, leading to yield reductions of up to 70%. The emergence and spread of devastating rice diseases pose a significant threat to global food security. Among these, bacterial leaf blight (BLB) is a major concern in Malaysia, attributed to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). BLB has been documented to cause yield losses ranging from 50-80% in susceptible rice varieties (Mohd Din et al., 2023). To date, there are no rice cultivars or varieties identified in Malaysia that demonstrate resistance to *Xoo* bacteria. Therefore, this underscores the necessity of implementing a strategy for early detection of this devastating disease.

The conventional method for detecting rice diseases typically involves recognizing symptoms by experienced plant pathologists or farmers. However, this approach lacks scientific validation. Other laboratory-based techniques, such as agar plate culture, polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA), while highly specific, still require trained operators for operation. Additionally, these methods are generally unsuitable for on-site analysis. Once symptoms of rice diseases begin to appear, the infection stage is considered late, and the widespread spread of the disease is inevitable. Timely detection of rice diseases plays a crucial role in effective plant disease management strategies. Recently, the biosensor technique has gained attention in rapid detection due to its sensitivity, portability, and versatility in detecting various analytes (Fang & Ramasamy, 2015; Umapathi et al., 2022).

In addressing rapid and early detection of rice diseases, an antibody-based electrochemical biosensor for the detection of *Xoo*, the causal pathogen of BLB disease, has been developed. Polyclonal antibodies against *Xoo* were produced in-house and used as a novel bioreceptor for detecting the target analyte. In accordance with the goals set forth in the National Agri-Food Policy 2021-2030 (DAN 2.0), which seeks to promote agriculture via digitalization and smart farming methods, the incorporation of the Internet of Things (IoT) enables the deployment of intelligent and precise agricultural systems. Leveraging IoT technology for advanced and early detection of rice diseases enables timely intervention and assists in reducing the spread and severity of infections. The application of the biosensor detection system on a portable device has been successfully performed on-field in BLB hotspot zones in the Integrated Agricultural Development Area (IADA), Selangor Northwest (Razali et al., 2022).

In the realm of technological digitalization, significant advancements have been achieved with the biosensor system for rice disease detection, including the development of the MARDI*sense* app for analysis and a web server for cloud computing. This report emphasizes the deployment of the new biosensor-IoT system at the MADA Smart Farming Rice Field to facilitate early detection of BLB. The purposes of the study are to evaluate the efficacy and performance of the integrated system under diverse conditions and to initiate routine monitoring for real-time early detection of rice diseases. The analyzed data were stored on the cloud server and are accessible from a dedicated webserver. This feature will benefit supervisors or extension agencies for real-time and routine monitoring.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals of analytical grade were sourced from Sigma-Aldrich, Japan, unless otherwise stated. Polyclonal antibody against *Xoo* was developed in-house at Biotechnology & Nanotechnology Research Centre, MARDI (Animal Ethics Committee approval number 20171103/R/MAEC27). Functionalized multi-walled carbon nanotubes (MWCNT-COOH) were from Dropsens, Spain, and the EZ-Link™ Plus Activated Peroxidase Kit was from Thermo Scientific, USA.

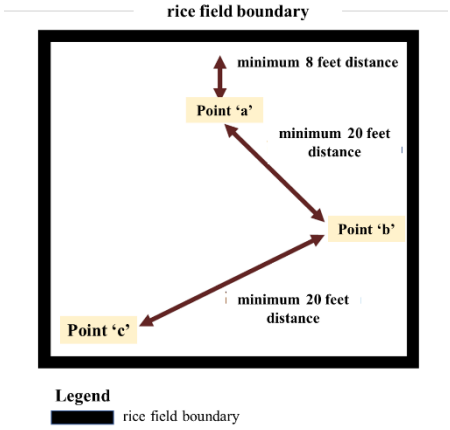
Preparation of Antibody-Modified Screen-Printed Carbon Electrodes (SPCEs)

Screen-printed carbon electrodes (SPCEs) used in the study were purchased from Biogenes Technologies, Malaysia. A single strip of the carbon-based SPCE has three (3) channels with a 4-mm combined working, counter and reference electrodes on a circular well with an 8-pin contact type. Surface modification of SPCEs was achieved by one-step electropolymerization as described by Razali & co-workers (2022).

On-site Sampling Procedures

The sampling location was designated within the IR4.0 Paddy Field Project at Kg. Bohor, Kedah, with coordinates 6°12'56.6"N 100°23'16.8"E. Ten (10) sampling points were established, each with three replicates, spanning four plots covering a total area of 42 hectares. The rice variety utilized in the sampling plots was MR297; rice leaves were collected and analyzed precisely at 32 days after sowing (32 DAS). Replicates were taken according to the crop cutting testing (CCT) technique (Figure 1). The first replicate, labelled as 'a,' was acquired approximately 8 feet (2.44 m) from the rice field boundary, while the subsequent replicates, designated as 'b' and 'c,' were collected with a separation of 20 feet (6.096 m) between them.

Figure 1. Points of replicates (*n=3*) adopted from crop cutting testing (CCT) (not to scale)



Sample Preparation and Analysis

Rice leaves were cut into 0.5 cm lengths, filling a quarter of the 5 mL Eppendorf tubes. A 1 mL sample buffer was added into the tubes and samples were ground using plastic pestles. Subsequently, a 10 µL blocking buffer of 0.1% ethanolamine was applied on the SPCEs surface, covering the working electrode (WE) area and left at 15 minutes at room temperature (RT). The electrodes were then washed with 1 mL washing solution, rinsed with distilled water and air-dried. Following this, a 5 µL of the previously extracted sample mixture was applied on the SPCEs and left to incubate. After 15 minutes, the SPCEs were washed, rinsed and air-dried. Next, 5 µL of the anti-*Xoo* antibody-conjugated horseradish peroxidase (HRP) solution was applied on the SPCE and left for an additional 15 minutes. The electrodes were washed and rinsed again before adding a final two drops of *3,3',5,5'*-Tetramethylbenzidine (TMB) substrate to the electrodes. The SPCEs were then inserted into the portable reader, and currents were measured, with results displayed on the Android device.

Enzyme-Linked Immunosorbent Assay (ELISA)

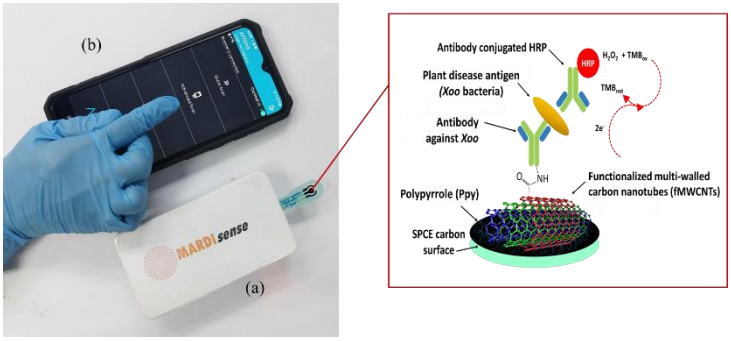
A Nunc™ 96-well microplate (Thermo Fisher Scientific) was initially pre-coated with the extracted samples and 0.1 M carbonate-bicarbonate buffer, pH 9.6. The latter was used as a negative control. After overnight incubation at 4ºC, the microplate was washed three times with Phosphate-Buffered Saline containing 0.05% Tween® 20 Detergent (PBST) to remove any unbound samples. Subsequently, anti-*Xoo* antibodies at a 1:1000 dilution was added to each well, followed by a 1-hour incubation at room temperature. The microplate was then washed again and blocked with 0.05% dry milk for one hour. Excess blocking buffer was removed through a repeat washing step. Next, a secondary antibody (i.e. anti-rabbit alkaline phosphatase conjugate) was introduced into each well and left for an hour. Before adding the substrate, any unbound secondary antibody was removed by washing the plates. A substrate solution (*p*-nitrophenyl phosphate, PNPP) was added, and the microplate was incubated for a suitable duration (typically 15-30 minutes) at room temperature, shielded from light. Following incubation, absorbance was measured at 405 nm using a microplate reader. The resulting data were analyzed to determine the presence and concentration of bacteria (specifically *Xoo* antigen) in the sample.

RESULTS AND DISCUSSION

Description of the Biosensor System

The developed biosensor system comprised of antibody-modified screen-printed carbon electrodes (SPCEs), a portable electrochemical reader connected to an Android device with pre-installed MARDI*sense* app. The portable reader has dimension of 11cm (L) x 6 cm (W) x 2.5 cm (H) and connected to a mobile phone via bluetooth integration (Figure 2). The sensor development employed a sandwich ELISA format (Figure 2, inset) as described elsewhere (Mohd Said et al., 2023). Initially, polyclonal antibody against *Xoo* was immobilized on the modified sensor strip surface, capturing the antigen (*Xoo* bacteria). Subsequently, a secondary antibody with a horse radish peroxidase (HRP) enzyme label, exhibiting affinity to another site of the antigen, was introduced. Electrochemical detection utilized 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB)/H_2O_2 as the enzyme mediator/substrate system. The current generated from the enzyme labelled antibody with its substrate (TMB) was recorded and measured accordingly via chronoamperometry technique at a fixed set potential.

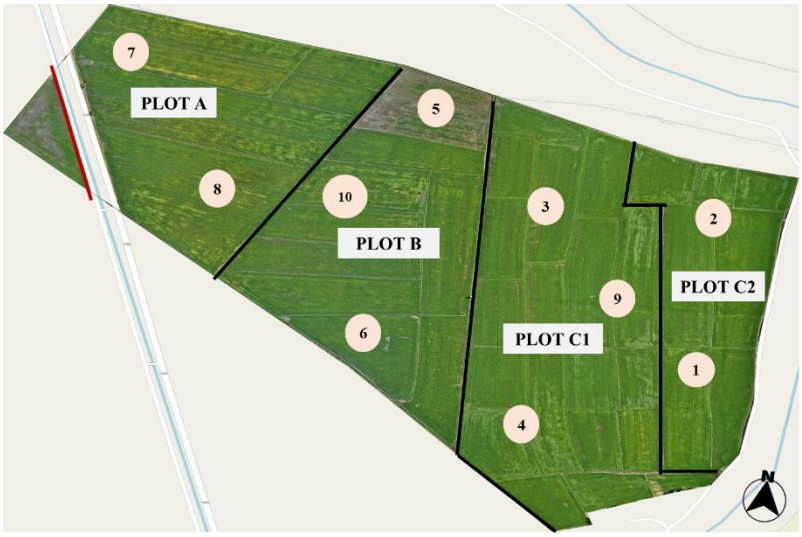
Figure 2. Components of the biosensor system: (a) A portable electrochemical reader with antibody-modified SPCE; and (b) An Android device with pre-installed MARDI*sense* app. *Inset right*: A modified SPCE surface for *Xoo* detection



Sampling Results

A total of 28 samples were collected from 10 sampling points within the MADA IR4.0 Paddy Field Project at Kg. Bohor, Kedah, covering approximately 42 hectares across four plots. As illustrated in Figure 3, two points were sampled from Plot A, three points each from Plot B and Plot C1, and two points from Plot C2. Three replicates (designated as a, b, and c) were taken from each sampling point, except for Point 6 in Plot B, which had only one replicate due to the rice plants being at 20 days after sowing (20 DAS) during the sampling period.

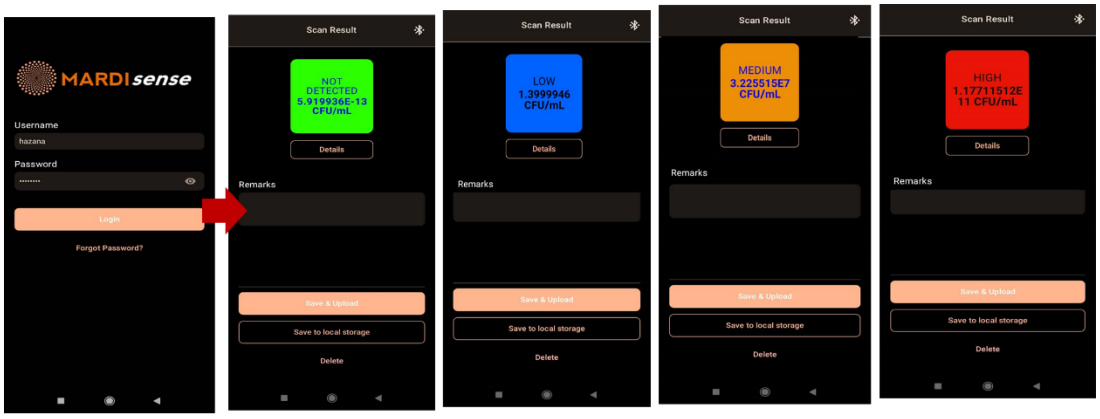
Figure 3. Location of the sampling points in MADA IR4.0 Paddy Field, Kg. Bohor



The analyzed results were interpreted and categorized as 'Not Detected,' 'Low,' 'Medium,' and 'High,' indicating the level of disease incidence based on the threshold set for bacterial concentrations associated with the disease (Figure 4a). Following the analysis, 26 out of 28 samples (92.86%) showed no incidence of BLB disease. Only two samples exhibited a low incidence of BLB, with bacterial concentrations less than 10^2 CFU/mL—specifically, sampling point 7b from Plot A and point 10c from Plot B. The results were directly uploaded to a cloud server through the website link https://biogenestech.net/MARDI_base/. Accessible to authorized administrators from this dedicated web server, various key parameters—including date, time, sampling location with GPS coordinates, analysis details with raw data, graphs, and data interpretation—are displayed for further reference (Figure 4b). This feature serves as a valuable tool for dashboard development and to aid in creating predictive models for future disease occurrences.

Figure 4. (a) Android interface for analysis and results interpretation based on bacterial concentration; and (b) Real-time data on the website, displaying sampling locations and details such as date, time and results for the sampling

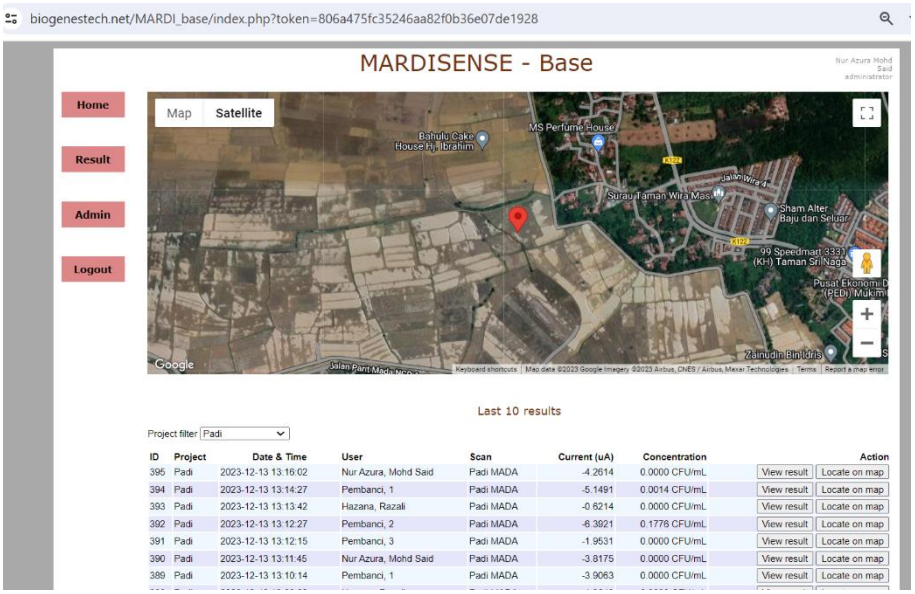
(a)



Authorized user log-in to begin analysis

Results are displayed and interpreted based on a bacterial concentration threshold

(b)



Subsequently, a validation study was conducted using the ELISA assay on the tested samples to verify the biosensor method. This involved a thorough evaluation of the sensor's performance and accuracy in detecting the pathogen causing rice disease. The primary goal of the validation study was to provide strong evidence of the sensor's effectiveness and suitability for precise and reliable analyte detection in practical scenarios. The ELISA method was specifically selected due to its use of an antibody-based format detection, similar to the biosensor system used in the field analysis.

According to Table 1, the ELISA method demonstrated a 100% incidence of free BLB disease, which aligns well with the 26 samples analyzed using the biosensor method. Notably, both sampling points 7b and 10c exhibited low bacterial concentrations of 30.88 CFU/mL and 1.975 CFU/mL, respectively. Although ELISA did not detect the bacteria in these samples, it is important to highlight that ELISA has a detection limit of 10^4-10^5 CFU/mL, whereas the immunosensor has a higher detection limit of 10^2 CFU/mL (Awaludin et al., 2020; Mohd Said et al., 2023). These findings highlight the potential advantages of using biosensor technology for more sensitive and accurate detection of bacterial pathogens, particularly at lower concentrations that may be missed by conventional ELISA methods.

Table 1: Correlation results for samples analyzed with biosensor-IoT system and ELISA method

Sampling Point	Plot	Biosensor-IoT	ELISA
1a	Plot C2	*ND	ND
1b		ND	ND
1c		ND	ND
2a		ND	ND
2b		ND	ND
2c		ND	ND
3a	Plot C1	ND	ND
3b		ND	ND
3c		ND	ND
4a		ND	ND
4b		ND	ND
4c		ND	ND
5a	Plot B	ND	ND
5b		ND	ND
5c		ND	ND
6a		ND	ND
7a	Plot A	ND	ND
7b		Low	ND
7c		ND	ND
8a		ND	ND
8b		ND	ND
8c		ND	ND
9a	Plot C1	ND	ND
9b		ND	ND
9c		ND	ND
10a	Plot B	ND	ND
10b		ND	ND
10c		Low	ND

*ND=not detected

CONCLUSION

Prioritizing early control measures for disease management is crucial to ensure the sustainability of rice production and meet the country's target of achieving 80% self-sufficiency level (SSL) by 2030. Disease management systems through the application of rapid detection technology with IoT integration are capable of bringing about systematic and efficient monitoring of rice disease attacks. Early identification not only assists in applying specific treatments but also encourages the adoption of preventive strategies, such as utilizing resistant crop varieties or optimizing cultivation practices. By incorporating these technologies and approaches into agricultural systems, we can boost the resilience of rice crops, reduce yield losses, and promote sustainable food production.

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