（学位第３号様式）

学 位 論 文 要 旨

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題　目	Research and Development of Early Detection and Control Methods for Anthracnose Agent (植物炭疽病菌の早期検出と防除に関する研究)

Plant diseases represent a threat to global food security, directly affecting food production, thus human wellbeing, and all the financial burden that comes with it. Early and quick detection of pathogens is crucial in managing spread of infections in biomedical, biosafety, food and agriculture fields. Being the causal agent of anthracnose, *Colletotrichum gloeosporioides* is one of the most destructive, widespread, severe, and economically important plant pathogens for tropical and subtropical crops. The fungal disease has an extensive distribution worldwide, and affect a wide variety of crops including cashew, bean, chili, passion fruit, mango, avocado, coffee, strawberry, tea, apple. In the present study, we: (i) developed a biological sensor for an early and rapid detection of *C. gloeosporioides* on farm and, (ii) investigated and proposed some appropriate control responses for the target pathogen.

In the first part of this work, biosensor technology, which is one of the most selective and sensitive for detection of pathogens/diseases, was used to fabricate a paper-based immunosensor for *C. gloeosporioides*. Hapten-labelled DNA amplicons (biotin and FITC) were used to interact specifically with corresponding antibodies: primary (anti-FITC) and secondary (anti-biotin), respectively. This antibody-DNA-antibody sandwich format provided a sensitive and specific detection platform for the target. Indeed, specificity of the fabricated immunosensor was tested against *C. theobromicola*, *C. candidum* and *Aspergillus fumigatus*, which attests to the reliability of our biosensor. The sensitive, specific and portable sensor was combined with an in-house-developed simplified DNA extraction on paper, and an isothermal amplification method to assure a complete outdoors detection of the fungus of interest. Our results offer a simple, quick, easy, and minimally-instrumented toolkit for *C. gloeosporioides* detection. It is scalable and adaptable.

In the second part of our research, copper metal was used as a control agent due to its diverse antimicrobial properties known for centuries. Our studies showed that nanoforms of copper had significant antifungal activities against our target fungus, with copper nanoparticles (Cu-NPs) offering the most sustainable efficacy and being more effective than its oxidative form (copper oxide nanoparticles: CuO-NPs). Scanning Electron Microscopy (SEM) images of the treated pathogen show that the hyphae had a swollen appearance, lost their filamentous structure, and the mycelia had a powder-like structure, indicating the probable destruction of the hyphal tubular cell wall. X-ray Difractogram (XRD) outputs showed substantial changes in the physical characteristics of the Cu-NPs after interaction with the fungus. Furthermore, Fourier Transform Infrared (FTIR) and biomimetic membranes studies were conducted to deepen our understanding of the copper nanoparticles control mechanism.

To the best of our knowledge, the present work is the first (i) to develop a paper-based biosensor for detection of *C. gloeosporioides*; (ii) to propose a simplified method with low-cost reagents that are compatible with LAMP towards on-farm detection; (iii) to report the way nanostructured copper forms inhibit/destroy *C. gloeosporioides* growth and viability; and (iv) to demonstrate chemo-physical changes in the copper nanoforms upon interaction with the pathogen. This opens new insights for further studies on the mechanism of copper's antifungal properties.