OPPORTUNITIES FOR USE OF BIOSENSING IN AGRICULTURAL QUARANTINE

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2nd ASEAN Diagnosticians Forum

Centara Grand Hotel at Central Ladprao, Bangkok, Thailand

20–21 August 2019

Biosensors For Plant and Animal Pest and Diseases Detection

- ✓ Plant pathogen detection is important as first step to manage a plant disease in greenhouses, field conditions and **at the country border**.
- ✓ Current **immunological techniques** used to detect pathogens in plant include ELISA and direct tissue blot immunoassays (DTBIA). **DNA-based techniques** such as PCR and real time PCR for pathogen identification and detection.
- ✓ However these methodologies are **time-consuming and require complex instruments**, being not suitable for in-situ analysis.

One of the main risks of fresh **fruit trade (exported and imported)** is the presence of fruit fly larvae in fresh fruit. The existence of these larvae has the potential to develop into adults and **infest fresh fruit products in destination countries**, especially in Indonesia.
Detection by Morfologyand Biomolecular

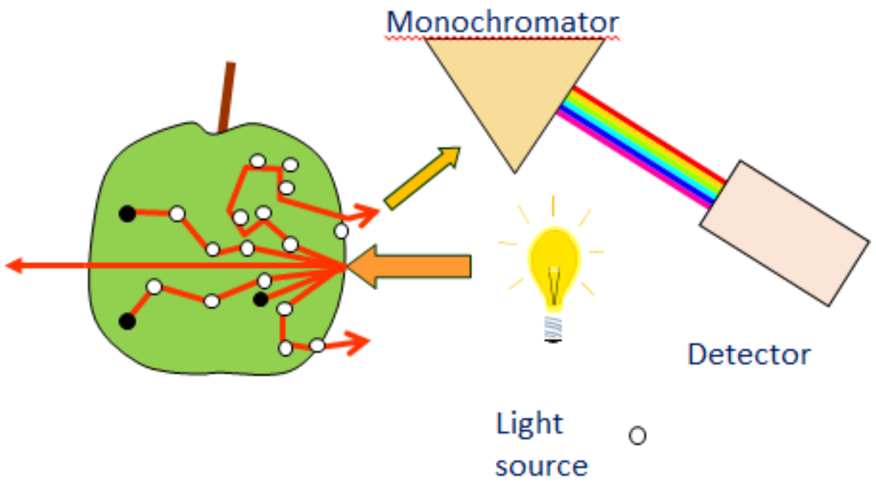
There is strong interest for developing **biosensing systems** for **early detection** both for plant and animal pest & diseases with high sensitivity and specificity.

Biosensors Methods

- Near Infrared Spectroscopy
- Fluorescence (FL spectroscopy & FL Imaging)
- **Ultrasonic (Proposed by Center Diagrostic laboratory of IAQA)**
- Thermal sensor
- Remote sensing
- Electrochemistry and photonic
- Imaging sensor

Near Infrared Spectroscopy

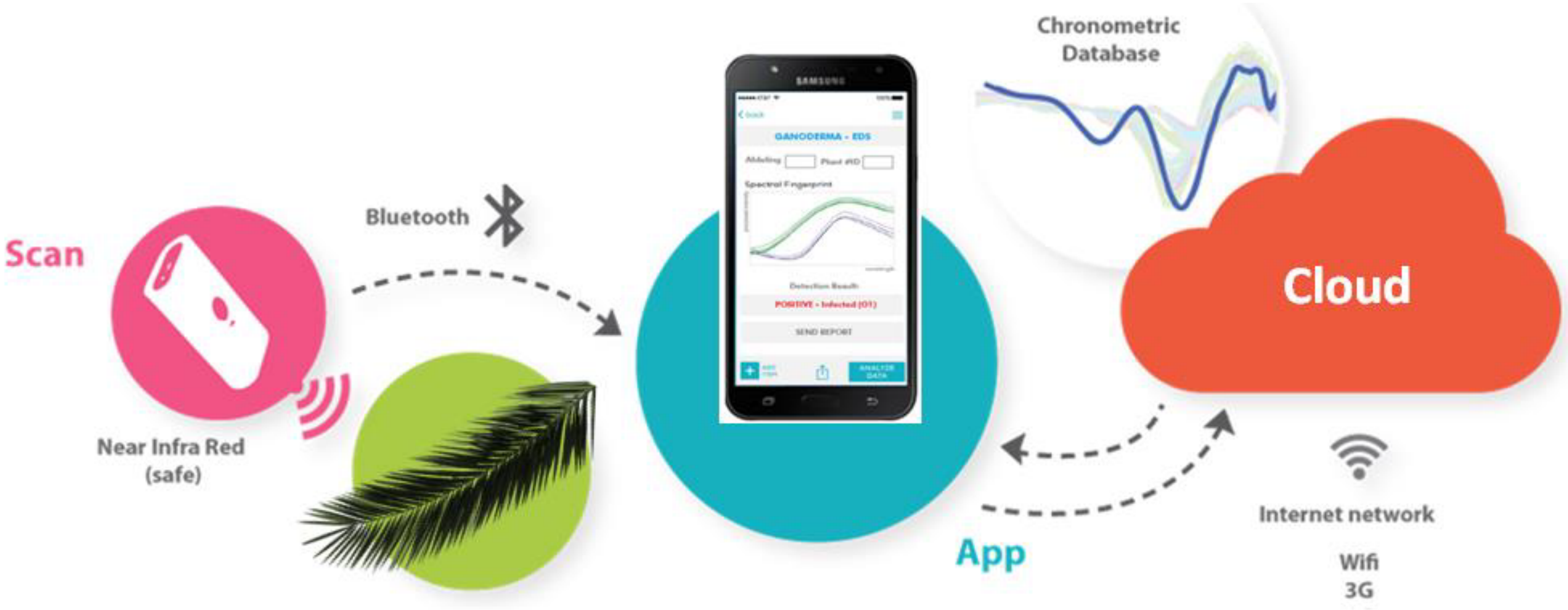
- NIR works which **specific organic molecules** absorb **specific wavelengths** of NIR (near infrared) light energy.
- The absorptions are directly **correlated** with the **concentration of the organic molecules.**
- The NIR is dependant upon the wet chemistry methods to attain the linear relationship between the molecular absorptions and the actual constituent concentrations.

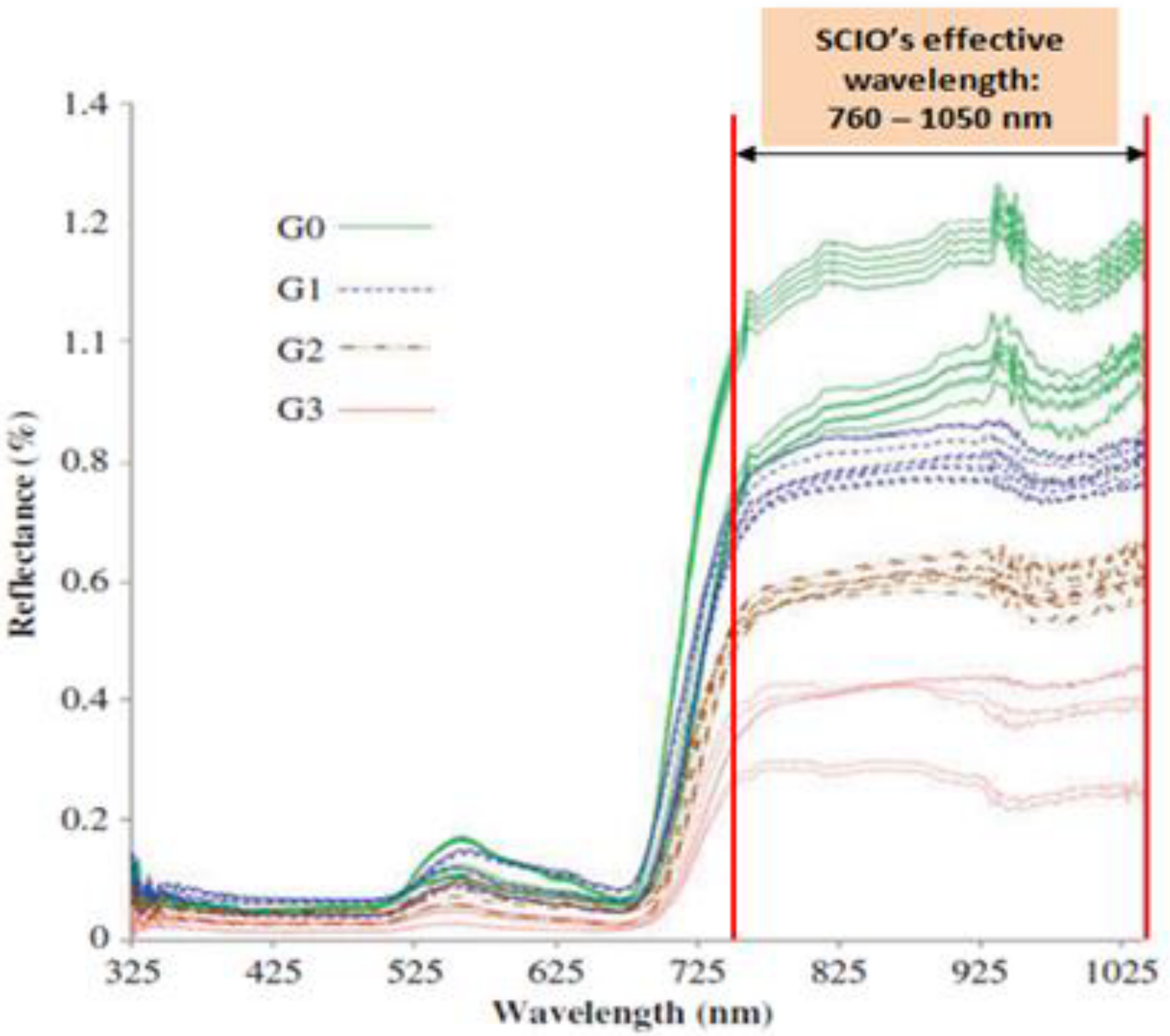


Near Infrared Spectroscopy Advantages :

- ➢Real-time analysis
- ➢Measure multiple constituents simultaneously
- ➢Non-destructive testing
- ➢No chemical waste stream, environmentally friendly
- ➢Less sample waste
- ➢Decrease operation costs

Fast prediction of chemical content non destructive based by NIR spectroscopy Near Infrared





Representative spectral signatures of healthy (G0) and Ganoderma-infected (G1, G2, and G3) leaf samples. (Liaghat*et al., 2014).*

Table 1 Examples of studies on plant disease detection using spectroscopic techniques.

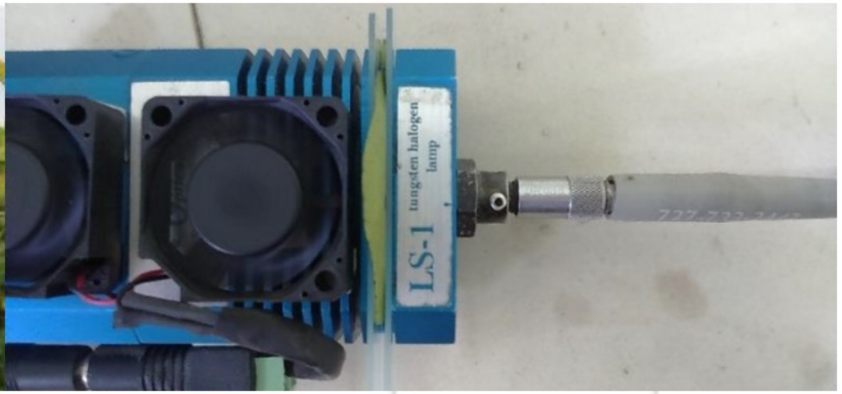
Plant	Disease/ Damage	Statistical Methods	Optimum spectral range	Reference
Citrus	Citrus canker	--	452, 685 and 735nm	Belasque et al. (2008)
Rice	Infested with brown planthopper	-	737–925nm	Yang and Cheng (2001)
Wheat	Powdery mildew and take-all disease	Analysis of variance, correlation and regression analysis	490nm to780nm, 510nm to780nm , 516nm to1300nm and 540nm to1300 nm	Graeff et al. (2006)
Rice	Brown planthopper and leaffolder infestation	Linear regression models	426nm	Yang et al. (2007)
Kiwifruit	Gray mold, Sclerotinia rot	Principal component analysis	-	Costa et al. (2007)
Wheat	Yellow rust	Regression	-	Huang et al. (2007)
Tomato	Leaf miner damage		800 to 1100 nm, 1450 and 1900nm	Xu et al. (2007)
Grapevine	Grapevine leafroll disease	Discriminant analysis	752, 684 and 970nm	Naidu et al. (2009)

Table 1. Application of Spectroscopy to fungi and mycotoxin measurements

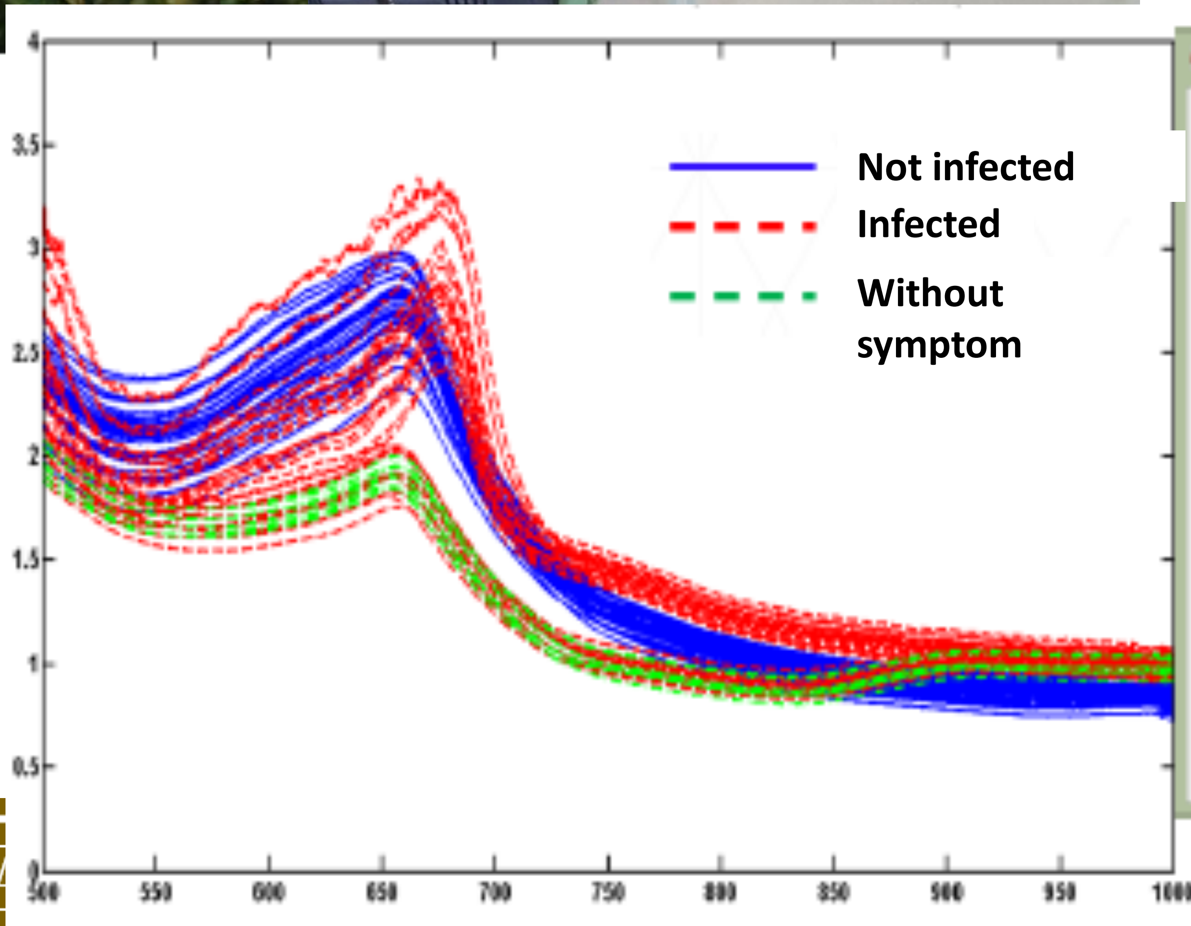
Technique	Sample	Detection	Wavelength/wavenumber range	Chemometrics	Year	Ref.
FTIR		Ochratoxin A	7,500–400 cm^{-1}	PLS	2013	[7]
Mid infrared FTIR-ATR	Corn	*Fusarium graminearum*	650–4,500 cm^{-1}	PCA, PLS, KNN[a)]	2003	[41]
Mid infrared FTIR-ATR, DR	Corn	*F. graminearum*		PCA, PLS, KNN	2004	[44]
FTIR-DRS, ATRS	Wheat	*F. graminearum*	650–4,000 cm^{-1}	MLR, PLS	2007	[1]
TIR, FTIR- PAS	Corn	*A. flavus*			1999	[24]
NIR, FTNIR	Maize, barley	Aflatoxin B1	400–2,500 nm, 1,112–2,500 nm	PLS	2009	[32]
NIR	Rice	*A. flavus* M3T8R4G3 aflatoxigenic strain	950–1650 nm	PLSR	2013	[69]
NIR	Maize	*F. verticillioides*	400–1100 nm		2005	[6]
NIR	Corn	fungal damage	900–1700 nm	LDA, MLP[b)]	2011	[72]
FTNIR	Maize	FB1 + FB2	650–2500 nm	PLS	2013	[22]
FTNIR	Wheat	DON	10000–4000 cm^{-1}	PLS, LDA	2014	[23]
FTNIR	Corn meal	FB1 + FB2	650–2500 nm	PLS	2012	[20]
NIRT	Wheat	DON	570–1100 nm	PLS, PCA	2003	[56]
Raman	Maize	Aflatoxins	785 nm	MLR, PCR, PLSR	2014	[44]
SERS	Maize	Aflatoxins	785 nm	MLR, PCR, PLSR	2014	[45]

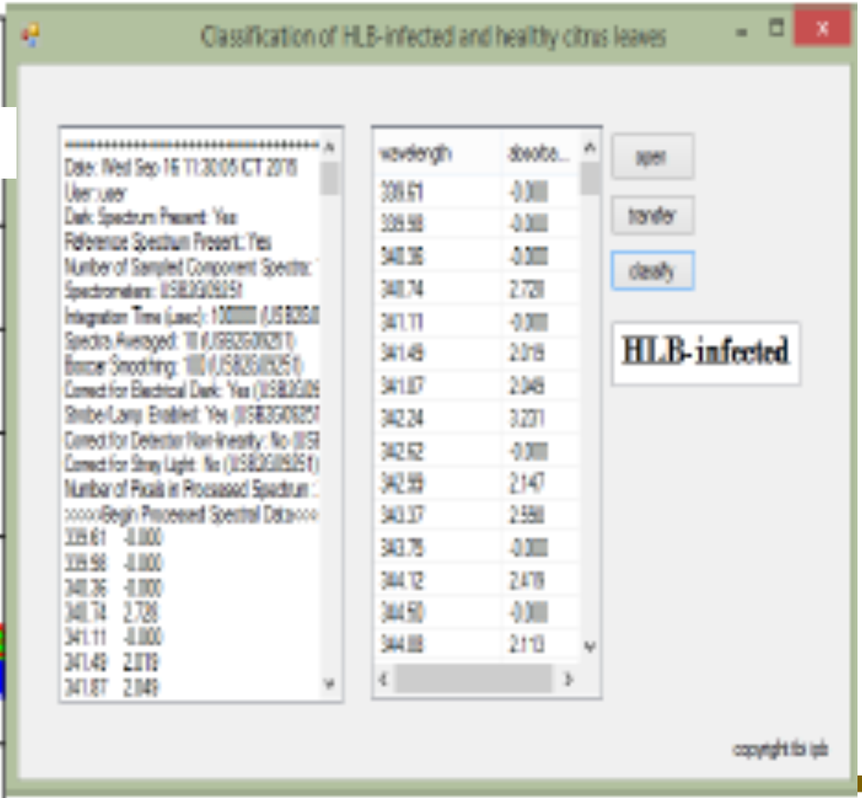
[a)] K-Nearest-Neighbor
[b)] Multi-Layer Perceptron

Visible near infrared spectroscopy for detection of Huanglongbingin citrus orchads



Spectroscopy Vis-NIR brand : OceanOptics.
Wavelenght : 339-1022nm.
The absorbance spectrum sample length at each measurement consisted of 2048 spectra

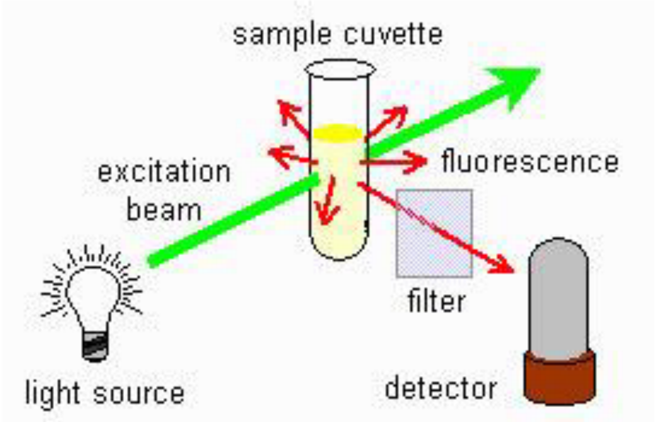


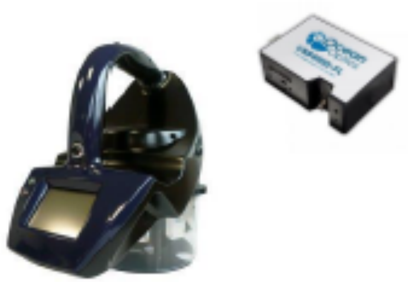


Some research have proven the ability of NIR spectroscopy for the detection of insects or insect damage in food commodities such as:

- blueberry (Peshlovet al.2009),
- cherry (Xing et al. 2008; Xing and Guyer 2008),
- fig (Burks et al.2000),
- green soybean (Sirisomboonetal. 2009),
- jujube (Wang et al.2010)

Fluorescence (FL spectroscopy & FL Imaging)

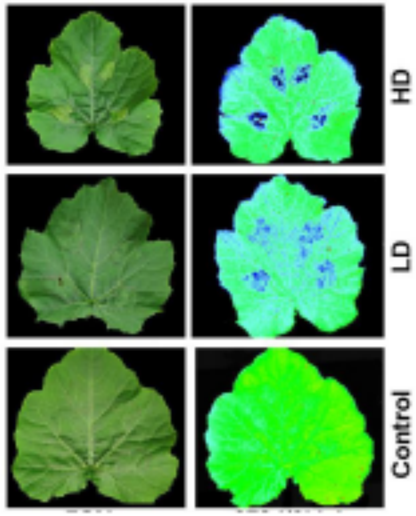




FL spectroscopy


Mould on citrus


Mould on pistachio nuts



Standard F440/F520 images of mock-control, LD, and HD- *D. dadantii* inoculated zucchini leaves

FL Imaging

Advantages :

•Very simple

•Specific to certain substances

•Is quite sensitive and can detect

•compound in low quantities

Disadvantages :

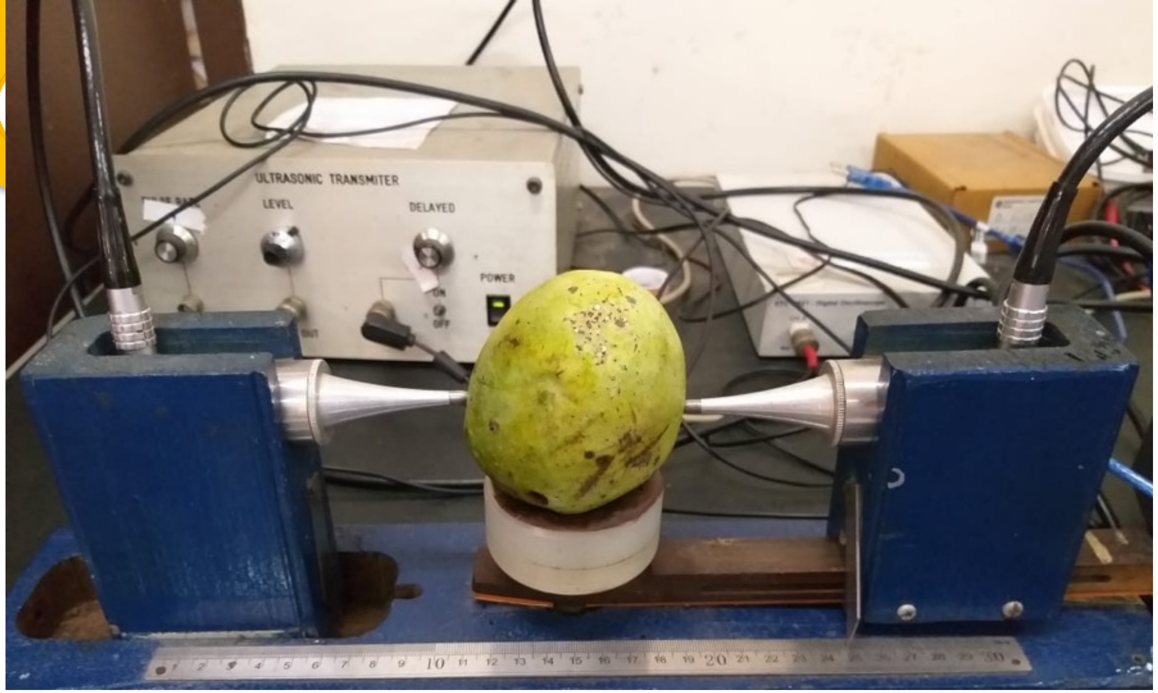
Not all substances can fluorescence so fluorescence-based technology is not always possible applied Excellence

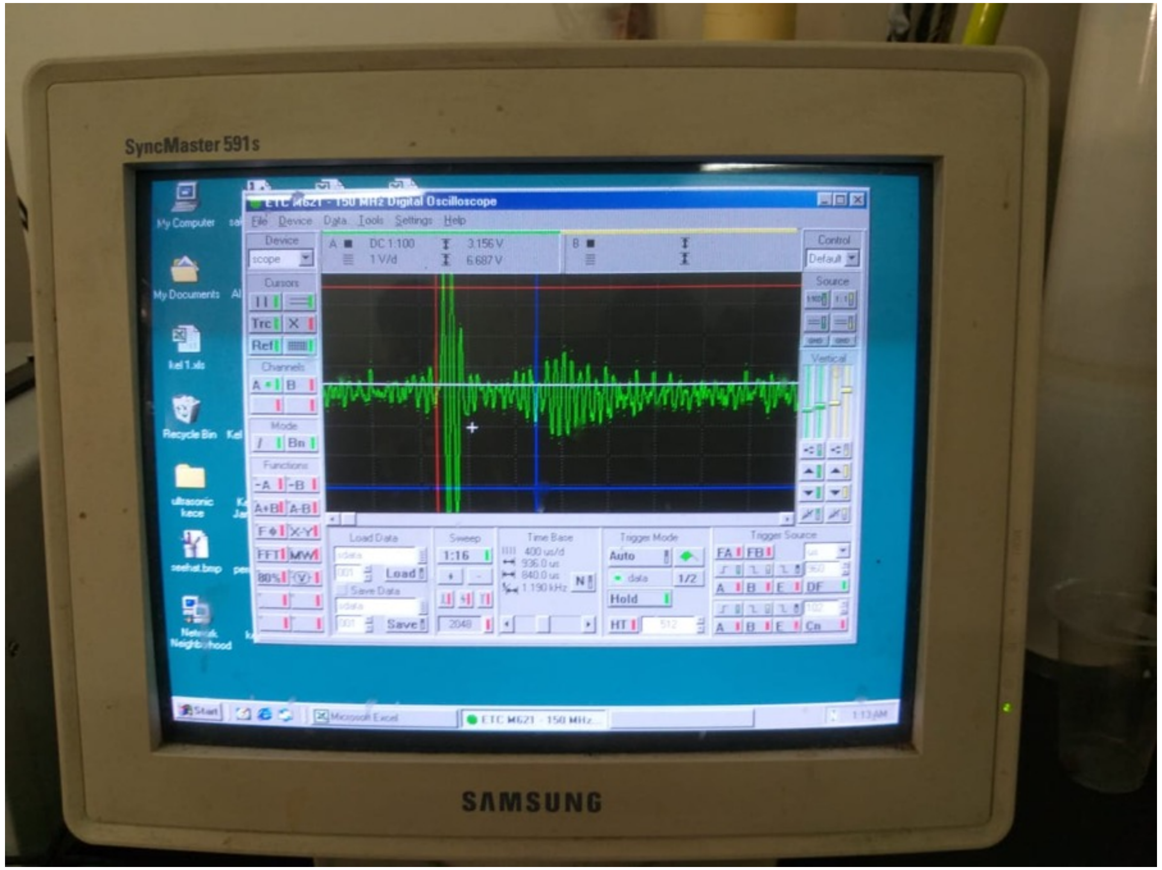
Ultrasonic

This technology is based on **the transmission of sound waves on the media** that are tested to then measure the propagation results of the energy emitted by the ultrasonic device.

Detection of fruit flies by Ultrasonic method

- Detection pattern: **measure the reflection of electromagnetic waves** generated by larvae and captured ultrasonic devices in the form of a magnitude graph
- Each fruit produces a different graph.
- Data analysis (transformation: Matlab program):
  - **Ultrasonic wave speed**
  - **Attenuation coefficient**
  - **Mo Value (Zero moment)**
- Summary : The relationship between fruit damage due to fruit fly attack with ultrasonic wave characteristics





The use of ultrasonic devices to detect fruit fly larvae is done by placing the fruit on an ultrasonic device then the waves propagated through the fruit are read one by one in computer software in graphical form as raw data.

The difference between **healthy fruit** and **infested fruit** in these parameters is measured based on **differences in media density (fruit)** which results in **different energy waves absorption,**

for example for liquid media has a greater attenuation coefficient than solid media,

so the rotten fruit attenuation coefficient should be more great compared to healthy fruit because rotten fruit texture is softer and nearly liquid.

IAQA Research Planning for Biosensors

No	Research	Current Method	sensor detection indicator	Sensor type
1	detection of plants affected by Quarantine pest (detection of decreased levels of secondary metabolites due to plants	Visual	Soil chemical properties, color response	Near Infrared (NIR) Remote/imaging sensing
2	detection of the presence of eggs and fruit fly larvae in imported and exported fruit	Visual (Morfology and morfomentry	Color response, fruit skin texture, level of rot	Sensor network and machine vision bioimaging (Fluorescence)
3	Detection of seed / seed / fruit / bulbs in the packaging	X ray	Color response	Fluorescence
4	Nematodes detection in garlic bulb using fluorescence sensor	Morfology and morfometry	Color response	Fluorescene, opto, novel sensor/electrochemistry and photonic, remote sensing
5	Detection of *Radopholus similis* from *Polyscias* sp. Root	Morfology and morfometry	Color response	Fluorescene, opto, novel sensor/electrochemistry and photonic, remote sensing
6	Detection of *Stenocarpella maydis of* import corn seed	Direct inspection, Morfology and morfometry	the response of color, temperaturand humidity	Imaging sensor, optical sensor (RGB, spectra sensor, thermal sensor, fluorescence imaging
7	Detection of *Microcyclus ulei* of indutry raw material from Brazil	Direct inspection, Morfology and morfometry	the response of color, temperaturand humidity	Imaging sensor, optical sensor (RGB, spectra sensor, thermal sensor, fluorescence imaging

Indonesia needs in this momentum

- Director General of Indonesian Agriculcure Qurantine Agency (**DG IAQA**) is very **concerned with biosensing**
- In this forum, we are looking for collaborative partners, for example :
  - Short training,
  - Scientific exchange,
  - Master or doctoral degrees, etc.

Thank you