**International Journal of Engineering & Scientific Research (IJMRA Publications)**
Vol. 6 Issue 5, May 2018,
ISSN: 2347-6532 Impact Factor: 6.660
Journal Homepage: http://www.esrjournal.com Email: esrjeditor@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

# Sensitivity and Selectivity Test of Pathogenic *Fusarium* spp. Associated with Wilt Fusarium Disease of Banana

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Article Received: 9th April, 2018
Article Revised: 18th April, 2018
Article Accepted: 29nd April, 2018

***Keywords:***

*Fusarium oxysporum* fsp. *Cubense;*
*pathogenicity test;*
*sensitivity test;*
*selectivity test.*

**Abstract**

One of the most important diseases of banana (*Musa paradisiaca* L.) in Indonesia is fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense*. Most farmer use banana's rhizome for their plantation, and the pathogen can be transmitted by this rhizome. A simple and effective method was needed to detect existance of Fusarium inoculum from banana plantation. The research aimed to make a simple, economic and effective methode in determaining existance of *Fusarium oxysporum* isolates by using banana's corms, taro's tubers and gingger's rhizomes. The pathogenicity test was done in 3 step, ie: necrotic test, sensitivity test and selectivity test. The pathogenicity test showed that banana corms could be used as a simple tool to recognized pathogenic *Fusarium* spp., seen from the necrotic symptoms that appear on pieces of banana corms. While taro's tubers and gingger's rhizomes could not be used as a tool, because there was no necrotic symptoms on it. Banana corms sensitivity enaugh to detect until 3.1 $10^3$ conidias of *Fusarium oxysporum* fsp. *cubense*. Banana corms not so selective to *Foc*, because it could detect some other Fusarium species, such ; *Fusarium* from taro, *Fusarium oxysporum* fsp. *cepae*, but couldn't detect *Rhizoctonia* sp., *Sclerotium rolfsii.*



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## 1. Introduction

Fusarial wilt of banana, caused by *Fusarium oxysporum* Schlecht *cubense* (E.F. Smith) Snyder & Hansen (1). The disease is quite widespread in banana regions of Asia, Africa, Australia, The South Pacific, and the tropical America. Fusarial wilt is one of the most catastrophic plant disease of the world, destroying more than 40.000 ha of bananas in Central and Soth America over a period of 50 years. In Indonesia, the disease was reported destroyed thousands of hectares of banana crops, both commercial banana plantations and banana folk cultivation (2) so this disease is still a major obstacle in banana cultivation and has spread from NAD to Papua (3).

Fusarium wilt disease in banana plants caused by fungus *Fusarium* f. sp. *cubense* (*Foc*) is an important and dangerous disease, because at the level of severe attack, this disease can cause death of banana crops and crop failure. These pathogenic fungi have high virulence, are polypagh and can survive in the soil for a relatively long time so that efforts to control this disease often have difficulty.

Until now there was no technology has been found to be truly effective and economically successful to control wilt disease. The difficulty of controlling the disease is partly because *Foc* is very specific and has diverse biological characteristics. *F. oxysporum* fsp. *cubense* consists of several races and strains with different levels of virulence, and has the ability to survive in land without the main host for up to 40 years (4). One of the alternative to prevent the occurrence of fusarium wilt disease is to detect the presence of *Foc* fungus inoculum on the land to be planted. It can be used as a preventive control measure.

## 2. Research Methods

This research was conducted experimentally in the laboratory using 28 isolates of *Fusarium* spp. fungi that have been isolated and observed by their characters, *Lasiodiplodia* sp., *Rhizoctonia* sp., *Sclerotium rolfsii, Fusarium* spp. from taro, *Fusarium* f.sp. *cepae* and *Fusarium oxysporum* f.sp. *cubense* TR4.

### 2.1 The Necrotic Test of *Fusarium* spp. on Banana Corms.

Necrotics test on the banana corms was done by placing each *Fusarium* spp. colony (Ø 0.5 cm) on each rhizome as many as 5 replications, then incubated for 7 days. For the control, the corms were not inoculated with the fungal inoculum. Observation of pathogenicity of isolates based on the presence or absence of necrotic on corms.

### 2.2 The Necrotic Test of *Fusarium* spp. on Taro Tubers.

Necrotics test on taro tuber was done by putting 3 taro tubers that have been cut according to eye buds with size 3 x 3 x 2 cm, and inoculated with *Fusarium* spp. colony (Ø 0,5 cm) on each tuber with 5 replications, then incubated during 7 days. For the control, tubers were not inoculated with fungal inoculum. Observation of pathogenicity of isolates based on the presence or absence of necrotic on taro tubers.

### 2.3 The Necrotic Test of *Fusarium* spp. on Ginger Rhizomes.

Necrotics test on ginger rhizome was done by putting 3 rhizomes of ginger that had been cut according to eye buds with size 3 x 3 x 2 cm, and inoculated with *Fusarium* spp. colony (Ø 0,5 cm) on each rhizome 5 replication, then incubated during 7 days. For the control, rhizomes were not inoculated with fungal inoculum. Observed pathogenicity of isolates based on the presence or absence of necrotic rhizomes.

### 2.4 The Sensitivity Test of Various Conidia Dilution of *Fusarium* spp. on Banana Corms.

Sensitivity test was performed on banana corms slice with size 6 x 6 x 5 cm. *Fusarium* spp. inoculum used first diluted from $10^{-1}$, $10^{-2}$, $10^{-3}$, to $10^{-7}$, then dripped on a piece of banana corm as much as 100 µl and repeated for each treatment as much as 5 times, then incubated for 7 days. Observation of pathogenicity of isolates based on the presence or absence of necrotic on banana corms.

**2.5 The selectivity Test of Banana Corms on Various Isolates of Soil Borne Fungi.**
Selectivity test was performed by inoculating the soil borne fungus (Ø 0.5 cm) on slices of banana corms, sized 6 x 6 x 5 cm. Each fungus isolate (*Lasiodiplodia* sp., *Rhizoctonia* sp., *Sclerotium rolfsii*, *Fusarium* spp. from taro, *Fusarium* f.sp.*cepae* and *Fusarium oxysporum* fsp *cubense* TR4) was inoculated onto banana corm slices, then incubated for 7 days. For Control, banana corms were not inoculated with the fungal inoculum. Observation of pathogenicity of isolates based on necrotic symptom on the corms.

**3. Results and Analysis.**

**3.1 The Necrotic Test of *Fusarium* spp. on Banana Corm, Taro Tubers and Ginger Rhizomes.**
Necrotic observations on banana, taro and ginger stumps were performed three days after inoculation up to 5 days after inoculation. The results showed that almost all isolates were able to cause necrotic effect on banana corms, but there were necrotic on taro tubers and ginger rhizomes (Table 1).

Table 1. Observation of Necrotic Test on Banana Corms, Taro Tubers and Ginger Rhizomes (5 DAI).

| No | Kode Isolat | Bonggol pisang | Umbi talas | Rimpang jahe |
|---|---|---|---|---|
| | | Kolonisasi dan Nekrotik | kolonisasi | kolonisasi |
| 1 | Bt3 | +[a] | - | - |
| 2 | Bt11 | + | - | - |
| 3 | Bt12 | ++ | - | - |
| 4 | Bt14A | + | - | - |
| 5 | Bt14B | + | - | - |
| 6 | Bt14C | + | - | - |
| 7 | Bt15 | + | - | - |
| 8 | Bt16A | ++ | - | - |
| 9 | Bt16B | ++ | - | - |
| 10 | Bt16C | + | - | - |
| 11 | Bt17 | + | - | - |
| 12 | BT28 | + | - | - |
| 13 | Bt29 | + | - | - |
| 14 | Bg1 | + | - | - |
| 15 | Bg2 | ++ | - | - |
| 16 | Bg4 | ++ | - | - |
| 17 | Bg6 | ++ | - | - |
| 18 | Bg7 | + | - | - |
| 19 | Bg10A | + | - | - |
| 20 | Bg10B | + | - | - |
| 21 | bg18 | + | - | - |
| 22 | Bg18c | + | - | - |
| 23 | A19 | ++ | - | - |
| 24 | A20 | ++ | - | - |
| 25 | A21 | ++ | - | - |
| 26 | Ta23 | ++ | - | - |
| 27 | Ta23a | + | - | - |
| 28 | Ta24 | + | - | - |

a = levels of necrotics symtomp (+ : low, ++ : medium, +++ : high)

**3.2 Sensitivity Test of Banana Corms to Various Conidia Dilution of *Fusarium oxysporum* f.sp. *cubense***

Sensitivity test was conducted to determine the ability of banana tree detection to the amount of Fusarium conidia. The initial density of *Foc* was 3.1 $10^7$ then diluted until $10^{-1}$ to $10^{-6}$. The result showed that the sensitive assay was up to $10^{-4}$ dilution. This means that banana corms coud still detect the presence of *Foc* inoculum up to the spore density of 3.1 $10^3$, as shown in Figure 1.

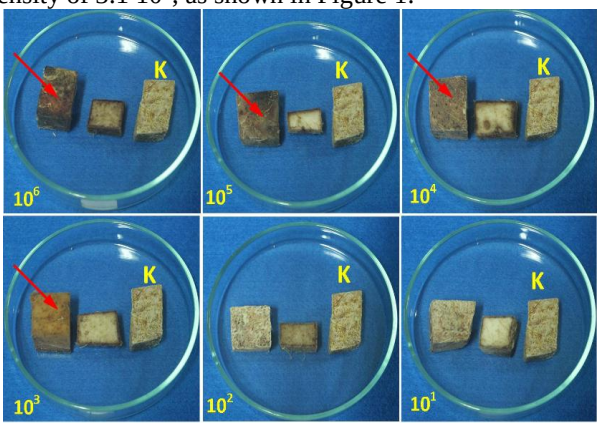


Figure 1. Cuts of bananacorms could detect *Foc* conidia density up to $10^3$ ( showed the change of color on the banana corms)

**3.3 The Selectivity Test of Banana Corms on Various Isolates of Soil Borne Fungi**

Selectivity test was performed to determine the ability of banana corms to detect other soil pathogenic isolates such as *Fusarium* oxysporum f.sp. *cepae*, *Fusarium* spp. from taro, *L. theobromae*, *Sclerotium rolfsii* and Rhizoctonia sp. Laboratory tests show that banana stumps can detect some soil pathogens other than Foc, such as, Fusarium spp. from taro and *L. theobromae*, whereas *Sclerotium rolfsii* did not cause necrotics on the banana corm, but could colonized the surface of the banana bunch tissue, whereas *Rhizoctonia* sp. unable to caused necrotism and colonized of banana tissue. This may indicated that banana corms could be used to detect some soilborne pathogens such as *Fusarium oxysporum* and *L. Theobromae.*

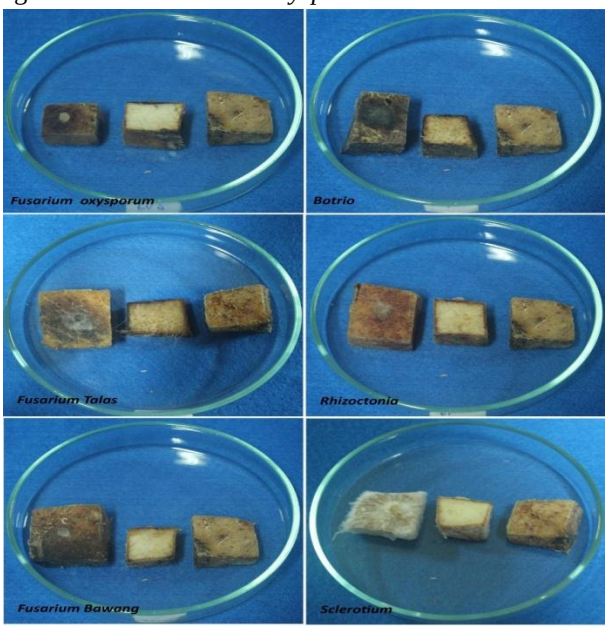


Figure 2. Selectivity test of various soil pathogenic fungi: A. *Foc* Race 4 (Control), B. Fusarium from taro, C. *Fusarium oxysporum* f.sp. *cepae*, D. *L. theobromae*. E. *Sclerotium rolfsii*, F. *Rhizoctonia* sp.

The results showed that banana corms were able to recognize the Fusarium isolate, shown by necrotic symptoms that appeared on the tissue of banana corms. *Fusarium* spp. which was placed on top of banana corms, able to recognize the compounds in the banana hump, so it was able to colonization and necrotics. (5) which uses onion bulbs for onion tuber detection of onions indicates that the necrosis caused by Fusarium infection in the basal plate may be an indication of pathogenic *F. oxysporum* infection in onion tubers. So this technique can be used as one of alternative pathogen testing carried by tuber on onion. the study states that *F. xysporum* carried by onion corms was not entirely pathogenic and nonpatogenic. Pathogenic fusariums can

not be distinguished by morphological observation, so further testing was needed to determine whether these fungi were pathogenic or nonpatogenic, one of the techiniques was using onion bulbs.
Test on taro tubers that occur only colonization, but does not occur necrotic on the tuber pieces, this is possible because taro is not a host of fungal isolates are tested. Even more interesting to the ginger rhizome, there was absolutely no colonization and necrotic. This indicated that the test isolates did not recognize the ginger rhizomes. It is suspected that the content of the compounds present in ginger was not favored by this isolate.
Cuts of banana corms have been able to detect conidia *Foc* race 4 to a density of $10^3$, indicating that this fragment was quite sensitive for *Foc* detection. The development of this method was expected to be applied to farmers' scale, to prevent the planting of bananas on infected land. In general, *Fusarium* spp. has a wide range of hosts and has a very wide variety of morphology and pathogenicity (6). Pathogenic and nonpatogenic strains of the Fusarium species can only be distinguished by pathogenicity testing, not by morphology or sexual compatibility studies (7).
In the selectivity test, in addition to being able to recognize *Foc* race 4, this piece of corms could also recognize other Fusarium derived from taro, and onion, and also could recognize other soil pathogens such as *Lasiodiplodia theobromae*, which is an isolate derived from the land of nutmeg rizosphere (Collection Susanna, IPB plant mycology lab.).

**4. Conclusion**

The development of a simple pathogenic Fusarium detection method using banana cultivation was a fairly accurate, relatively fast and sensitive technique although not so selective. The results showed that banana fragments can detect propagules of pathogenic Fusarium fungi that are 3-5 days after inoculation (DAI), characterized by the occurrence of necrosis in banana congestion tissue inoculated with Fusarium spp isolates. The detection method with banana corm's slices was sensitive enough to detect *Foc* inoculum up to 3.1 x $10^3$ conidium density. However, it was not selective between Fusarium species other than *Foc* such as *F.oxysporum* from taro, and *F.oxysporum* f. sp *cepae*. However, pieces of banana corms are quite selective against other soil-borne pathogens such as *Sclerotium* sp. and *Rhizoctonia* sp., except for the soil fungi *Lasiodiplodia theobromae*.

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