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# Biological Control of *Fusarium oxysporum* f. sp. *cubense* by *Pseudomonas fluorescens* and BABA *in vitro*

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**Abstract:** The effect of *Pseudomonas fluorescens* and the chemical fungicides β- aminobutyric acid to *Fusarium oxysporum* in banana disease has to the biocontrol solution of these bacteria. Under invitro conditions *Pseudomonas fluorescens* clearly inhibited *Fusarium oxysporum* f. sp. *cubense*. At a concentration of (50, 200, 250, 350) µg/ml BABA did not inhibited invitro germination of conidia and mycelium growth of FOC on PDA medium.

**Key words:**Biological control · BABA · *Fusarium oxysporum* f. sp. *cubense* · *Pseudomonas fluorescens* · Banana

## INTRODUCTION

Various synthetic and biological compounds have been described which are capable of controlling a large variety of plant diseases without displaying a direct antibiotic effect themselves. These substances are called inducers based on their ability to induce resistance in the treated plants. However, among the biocontrol agents, fluorescent *pseudomonas* and the Nonpathogenic isolates of *Fusarium oxysporum* on soil inhabitants and have been widely used as antagonists against fusarium wilt of several agriculture important crops [1]. Aminobutyric acid is anon-protein amino acid, which induced resistance against abroad range of disease causing organisms, including fungi, bacteria, viruses and nematodes [2, 3]. Only a few reports indicated that pretreatment with BABA can affect on wilt diseases by inducing systemic resistance against *Fusarium oxysporum* [4] that infect many plants for example in banana, tomato [5], melon [6] and watermelon [7]. It is also capable of inducing systemic resistance against numerous pathogens, including *Phytophthora infestans* [8], *Botrytis cinerea* [9], Tobacco mosaic virus [10], *Peronospora parasitica* [11], *Aphanomyces euteiches* [12], *Pseudomonas parasitica* [13], *Meloidogyne javanicum* [14], *Heterodera avenae* [15] and *Heterodera lalipons* [15].The aim of study of the research was performed as a first step toward the development of biological control for the management of fusarium banana wilt diseases.

## MATERIALS AND METHODS

**Isolates *Fusarium oxysporum:*** The banana wilts pathogen *Fusarium oxysporum* was isolated from wilted banana (Terong - Perak - Malaysia) plants using half strength potato dextrose agar (PDA) medium. the culture was maintained on carnation leaf agar medium [16] for immediate use and for long term use, the culture was stored as dried filter paper cultures according to [17] at 4°C.

**Isolation of *Pseudomonas fluorescens:*** *Pseudomonas flurescens* strain pa4 was isolated from the rhizosphere of banana with king's medium b (kmb) [18] and characterized through microbiology and biochemical tests as biotype III [19].

**Antagonism of Bacteria:** Discs (5mm) of 7days old mycelium of *Fusarium oxysporum* f. sp. *cubense* were placed in the center of plats with PDA. The colony of pseudomonas flourescens were placed equidistant sites 1cmfrom plate periphery. After 7days of inoculation at 25°C the percentages of antagonistic colonies of bacteria inhibit growth of *FOC* mycelium in the whole population was assessed [20-22].

**Method of the Filtrates of Culture:** Suspension of *Pseudomonas fluorescens* was inoculated with n. broth for each experiment; flasks in triplicate were incubated at

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30°C for 48h. The filtrate culture of bacteria were removed by centrifuging the cultures at 5000rpm for 30 minute the supernatant was used immediately [21], about 1ml of filtrate culture of the bacterial was added in PDA, then disc of *FOC* was placed in center of petri plate containing PDA and filtrate culture. Plates were cultured for 7days at 28°C and fungal growth was measured and compared to control growth where the filtrate culture was replaced with sterile distilled water.

**Selection of Protease Positive *Pseudomonas* Strain:** Proteolytic activity was estimated in 48h pseudomonas supernatants from king B [18], TSB (difco), PDA [23]. The radial diffusion assay in skim milk agar plate agar [24] was used zones of clearing (mm) around wells were measured. The pH of media was checked before inoculation of bacterial strains and after their growth.

**The Effect of BABA on the Growth of *FOC In vitro*:** BABA was add in such quantities that the concentration were, respectively, 50, 200, 250 and 350 µg $cm^{-3}$, to autoclaved potato dextrose agar medium (PDA-Merck), after sterilizing and cooling to 45°C. The prepared solutions were poured onto 90 mm Petri dishes. Medium without BABA addition was the control. 5 mm disks of PDA medium overgrown with a 7-day culture of *Fusarium oxysporum* f. sp. *cubense* were transferred onto solidified medium in the middle of dishes. After 2, 4 and 6 days of incubation at a temperature of 25°C in dark, the diameter of the mycelium colony were measured in two perpendicular directions. For each concentration of BABA, the linear growth of the mycelium of the series at a weekly interval.

## RESULT AND DISCUSSIONS

In order to deal with the evaluation and effectiveness of BABA and PF on the growth of FOC, we give emphasize on how we executed and concluded the results. The FOC bacteria, was isolated banana rhizosphere samples were collected from random banana fields in Terong-Perak-Malaysia. Bacteria and fungi were isolated from the rhizosphere and root and soil samples of symptom wilt and non wilt banana plant. Twenty-Fife *Pseudomonas* spp. strain showing weak in inhibition zones against FOC on PDA were isolated from root and soil. Four out of seventeen *Pseudomonas fluorescense* showing high antifungal activity against FOC *in vitro* (Dual culture).

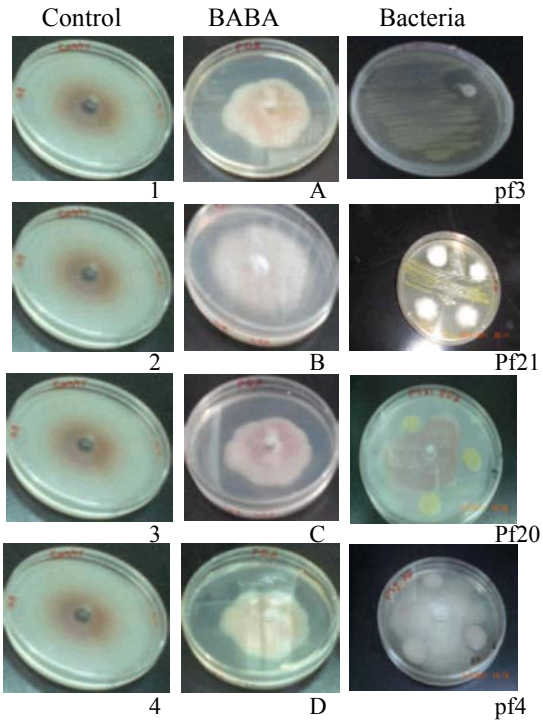


Fig. 1: The effect of BABA and *Pseudomonas fluorescense* on the growth and development of *FOC* on PDA medium *in vitro*.

The effect of BABA with different concentrations on the FOC *in vitro* is shown in figure 1. The result showed that the growth of *Fusarium oxysporum* f. sp. *cubense* is limited in about 50%, similar results are reported by other authors. The activity order is mostly the same as in higher and low concentration.

With respect to PF against *Fusarium oxysporum* f. sp. *cubense*, it can be easily recognized from Figure. 1 that the presence of PF is more effective with respect to the control sample in inhibiting the bacterial growth than BABA. This can be easily followed by measuring the area of the halo formation around the different disks. The PF can be arranged in the following order according to their efficiency as antimicrobial against FOC, PF3 >PF12 >PF20 >PF4. It can be noticed that PF4 show little activity in stopping the growth of FOC on the contrary to the rest of PF.

Table 1: Action of proteolytic *Pseudomonas fluorescense* on Skim milk

| Bacterial Strains | King B | | TSB | | PDB | |
|---|---|---|---|---|---|---|
| control | pH | Zone(mm) | pH | Zone(mm) | pH | Zone(mm) |
| | 7.5 | 0.00 | 7.5 | 0.00 | 5.0 | 0.00 |
| | 7.5 | 8.65 | 7.8 | 13.00 | 4.5 | 0.10 |
| | 7.7 | 24.30 | 8.00 | 0.00 | 4.1 | 0.10 |
| | 7.6 | 19.00 | 8.2 | 17.50 | 5.3 | 12.21 |

The find pH and zone of clearing around wells with Tested supernatants on skim milk plates as shown in Table 1.

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