

Short Communication: Biological control of *Fusarium* wilt on banana plants using biofertilizers

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Abstract. Widyantoro A, Hadiwiyono, Subagiya. 2020. Biological control of *Fusarium* wilt on banana plants using biofertilizers. *Biodiversitas* 21: 2119-2123. *Fusarium* wilt is an important disease of banana caused by *Fusarium oxysporum* f. sp. *cubense* (FOC). The FOC is a weak parasite that attacks many bananas whose conditions are weak such as nutrients. Therefore, controlling *Fusarium* wilt through fertilization is important so that bananas are not nutrient deficient which can cause plants to be susceptible to FOC. The research aimed to study the effect of biofertilizer applications on FOC suppression *in planta*. Seven treatments were tested on banana seedlings cv. Ambon Kuning under a completely randomized design: (i) no biofertilizer, (ii) carrier material of biofertilizer, (iii) comparative biofertilizer product, (iv) *Azotobacter*, (v) *Azospirillum*, (vi) *Streptomyces* and (vii) *Bacillus*. The present study showed that biofertilizer agents were antagonistic to *Fusarium* wilt. The results showed that biofertilizer agents had the potential to suppress the *Fusarium* wilt in planta. *Streptomyces* and *Bacillus* were the most effective in controlling the *Fusarium* wilt. *Azotobacter* and *Azospirillum* had not been able to prevent the incidence of wilt disease.

Keywords: Antagonism, *Azospirillum*, *Azotobacter*, *Bacillus*, *Fusarium oxysporum*, *Streptomyces*

INTRODUCTION

Banana cultivation without balanced fertilization tends to be susceptible to *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) (Ploetz 2006). Pathogens spread faster if fertilization is not enough for plants (Moore et al. 2002). FOC has been reported to infect almost all types of bananas and this disease is widespread in several tropical countries such as India, Indonesia, Malaysia, Thailand, Kenya, Brazil, Australia and Africa (Ploetz 1994; Davis et al. 2002). FOC Tropical Race 4 was reported to infect bananas in 19 of the 135 countries producing bananas (Ploetz 2015). FOC infects bananas in almost all regions in Indonesia including the latest report in Papua (Rusli et al. 2018). FOC has reportedly attacked banana plantations in Lampung, Kalimantan, Java, Lombok, and Sulawesi (Riska et al. 2012). The rapid attack of *Fusarium* pathogen on bananas is the basis of government regulations to prohibit the distribution of plant materials in an effort to prevent the spread of wilt (Zheng et al. 2018). The severity of *Fusarium* wilt of banana was controlled by up to 70-79% by using biocontrol agents and up to 42-55% by arbuscular mycorrhizal fungi and non-pathogenic *Fusarium* strains (Kalaiponmani et al. 2017).

Biological control of plant pathogens using biofertilizer agents is widely known because the agents can grow rapidly under various substrates and various environmental conditions (Rebib et al. 2012). Several biofertilizer agents such as *Azotobacter*, *Azospirillum*, *Streptomyces*, and

Bacillus can synthesize plant growth-promoting hormones, mobilize nutrients, and prevent plant diseases (Fraile et al. 2015). Several types of bacteria such as *Streptomyces* and *Bacillus* have been able to produce antibiotics found in colonized roots that are effective and can grow optimally in the rhizosphere (Cowan and Talaro 2006). *Azotobacter* was reported to produce *indole acetic acid* (IAA) as a growth booster and alginate as a cell protector (Auhim and Hassan 2013). *Azospirillum* was reportedly able to decompose soil organic matter and as soil aggregate stabilizer (Okon and Kalpunik 1986). Several studies have shown that *Azospirillum* effectively colonizes the rhizosphere. *Streptomyces* and *Bacillus* were reported to produce antifungal compounds (Prepagdee et al. 2008) and chitinase enzymes that can degrade *Fusarium oxysporum* cell walls (Pryor et al. 2006).

The function of biofertilizer was reported to increase soil fertility (Simarmata et al. 2016). Biofertilizers were expected to have multiple functions which include providing nutrients for plant growth and suppressing the pathogen especially *Fusarium oxysporum*. The mechanism of inhibition of biofertilizing agents against pathogens can be parasitism (hyperparasitism), antibiosis, competition, induction of host resistance, and plant growth promotion (Pieterse et al. 2014; Heimpel and Mills 2017). This research aims to compare the biocontrol efficiency of several strains of biofertilizer agents and commercial biofertilizers in the management of FOC in bananas under greenhouse condition.

MATERIALS AND METHODS

Research was conducted at greenhouse, Faculty of Agriculture, Universitas Sebelas Maret (UNS) in Surakarta, Indonesia. Suspension of biofertilizer agents such as *Azotobacter* (AA1), *Azospirillum* (AA2), *Streptomyces* (J1) and *Bacillus* (T1) was prepared by culturing them in 250 mL of King's B liquid medium. The liquid inoculant in the Erlenmeyer flask was shaken at a speed of 120 rpm for 5 days until the bacterial population reached 10⁷-10⁸cfu mL⁻¹. Comparative biofertilizer product used in this experiment contains *Azotobacter* (10⁷cfu mL⁻¹), *Azospirillum* (10⁷cfu mL⁻¹), *Streptomyces* (10⁵cfu mL⁻¹), and *Bacillus* (10⁸cfu mL⁻¹) applied to plant of 25 ml with a concentration of 10 cc L⁻¹ using the soil drench method. HNC (*high nutrient concentrate*) as an enrichment media contained molasses was diluted through a sterilization process from a concentration of 60% with a pH measurement of 4-5. Comparative biofertilizer and HNC was manufactured by PT Indo Acidatama Tbk, Indonesia.

FOC isolates were obtained from the fungal collection of Plant Pests and Diseases Laboratory of UNS. FOC isolates were tested for their pathogenicity on banana seedlings for a month before being used in the greenhouse study. Antagonism test *in planta* was carried out using banana plants (*Musa acuminata* AAA cv Ambon Kuning) aged two weeks post acclimatization by flushing biofertilizer agents of 25 mL in polybags a week before FOC inoculation. FOC suspension with density 10⁶ mL⁻¹ spores was injected into the corm of 5 mL.

A total of 7 treatments (T) was applied to banana plants which include: no biofertilizer (T1), HNC as a carrier material (T2), comparative biofertilizer (T3), *Azotobacter* (T4), *Azospirillum* (T5), *Streptomyces* (T6) and *Bacillus* (T7). The experiment was conducted in a completely randomized design (CRD). Each treatment (T) consisted of 3 observation units with 3 replications which made up to a total of 63 units of banana plants. The observation variables included: disease intensity (IP), infection rate (r), area under the disease progress curve (AUDPC), plant height, and wet biomass. Disease intensity (IP) was calculated as:

$$IP = \frac{\sum (n \times v)}{N \times Z} \times 100\%$$

Where; IP: Disease intensity (%); n: Number of leaves on each corresponding score; v: Score of disease on corresponding leaf; N: Highest score; Z: Number of observed leaves.

Area Under the Disease Progress Curve (AUDPC) was calculated as:

$$AUDPC = \sum_{i=1}^n \frac{X_i + 1 + X_{i+1}}{2} X(t_{i+1} - t_i)$$

Where; X_i: Disease intensity; t_i: Days after inoculation; n: Assessment dates (i = 1....n)

Infection rate (r) was calculated as:

$$r = \frac{2.3}{t} \left(\log \frac{1}{1 - X_t} - \log \frac{1}{1 - X_0} \right)$$

Where; r: infection rate (unit per day); t: observation interval (7 days); X₀: proportion of initial disease of observation; and X_t: proportion of diseases observed to t.

The data were analyzed with F test and Duncan's Multiple Range Test (DMRT) at level of 5% using SPSS 16 software.

RESULTS AND DISCUSSION

Disease intensity and infection rate

The results showed that several agents were antagonistic to FOC but some strains are not able to improve banana's tolerance against FOC (Figure 1). Banana cultivars used in the treatment are Ambon Kuning seedlings that are susceptible to FOC. *Azotobacter* and *Azospirillum* could not inhibit the infection of the pathogen. *Streptomyces* and *Bacillus* are the most effective in suppressing the infection of FOC. The ability of *Bacillus* to inhibit pathogens has been seen for two weeks after application in the field. Antagonistic agents could suppress the growth of pathogens through direct or indirect inhibitory mechanisms. Hadiwiyono et al (2013) added that antagonistic agents could inhibit the disease progression through the mechanism of competition, antibiosis, and plant growth promotion.

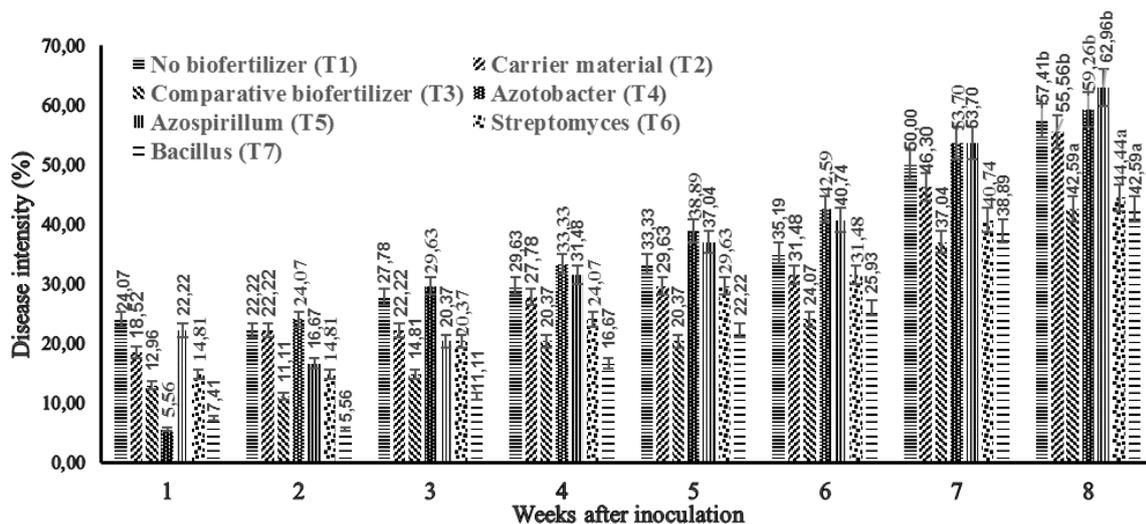


Figure 1. Disease intensity in potted banana plants inoculated with FOC

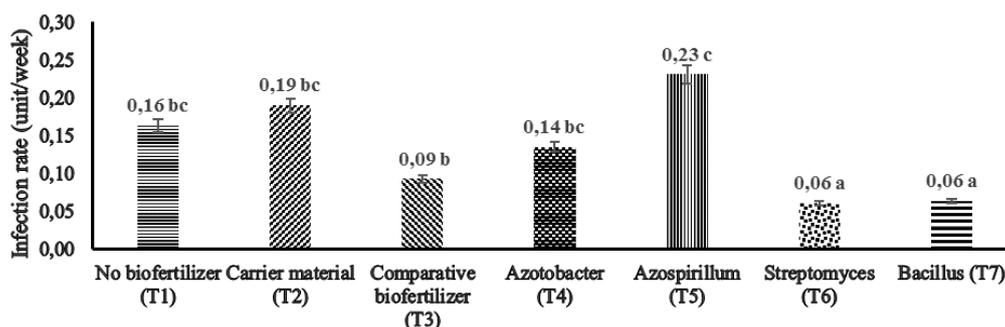


Figure 2. Infection rate in potted banana plants inoculated with FOC at aged eight weeks post acclimatization

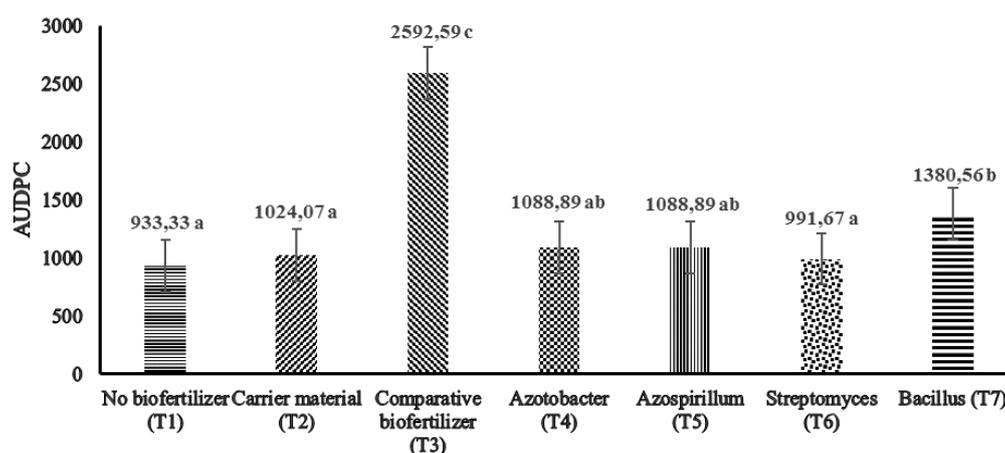


Figure 3. AUDPC in potted banana plants inoculated with FOC at aged eight weeks post acclimatization

Increasing the disease's intensity over time in banana plants showed that several agents could not prevent FOC infections, especially *Azotobacter* and *Azospirillum*. Comparative biofertilizer product with a composition of four isolates could be reduced the disease intensity. Widyantoro et al (2019) added that *Streptomyces* and *Bacillus* were able to inhibit FOC colony *in vitro*. *Azotobacter*, *Azospirillum*, *Streptomyces*, and *Bacillus* are among the antagonistic bacteria from the rhizosphere that are adaptive to extreme environments (Pelczar and Chan 1986). Susanna (2006) argued that the effectiveness of bacteria in suppressing pathogenic infections was strongly influenced by the ability of each agent to colonize the rhizosphere.

The results showed a decrease in infection rate after *Streptomyces* and *Bacillus* were applied compared to control without biofertilizer agents (Figure 2). Infection rate was related to development of diseases affected by environmental conditions. The infection rate was related to spread of the pathogen after penetration. Cv. Ambon Kuning banana treated with biofertilizer bacteria showed symptoms of wilt disease.

The appearance of wilting symptoms arose in two-week-old bananas after FOC inoculation. This could be seen in the infection rate at aged two weeks which seems to

decrease so that it showed suppression of pathogenic activity. In addition, a virulent pathogen would be faster to proliferation than biofertilizer agents. This happened because biofertilizer agents that were introduced a week before inoculation of pathogen with liquid formulation must be adapted to the environment. Ideally, it should be feasible to apply biofertilizers that could be added to the soil using mis-sprayers (Yardin et al. 2000; Mahdi et al. 2010).

Area under the disease progress curve

AUDPC showed that the spread of epidemic disease had experienced a significant increase (Figure 3). Biofertilizer tested especially those containing *Streptomyces* and *Bacillus* were being able to suppress FOC. Hadiwiyo et al. (2013) added that several *Bacillus* isolates were able to suppress FOC infection in planta, but not all isolates were capable of inducing the systemic resistance of banana plants in greenhouse.

During the whole experiment, the AUDPC of the untreated control banana plantlets was higher than those of the treated plantlets. Figure 3 showed a graph of the growth of *Fusarium* wilt more rapidly when there were no inhibiting agents. AUDPC values in each treatment showed a different area of the curve but at aged eight weeks after

inoculation showed similar to the disease severity. Vascular tissue in pseudo stems arose from brownish color. Antagonistic agents were usually disabled to control the wilt disease when it was already in the plant tissue (Hadiwiyono and Widono 2012). Although agents could induce the host plant resistance it could not be stopped the spread of pathogen in the plant tissue.

Biofertilizer is easy to reproduce, low cost, and do not damage the soil environment. However, introducing new microbial strains into the soil could result in competition for space and nutrients with the native strains in the soil. Biofertilizer agents with a density of 10^7 - 10^8 cfu ml⁻¹ in this study could be adapted to the environment. The high density could have supported the growth of bacterial colonies to compete with pathogens for eight weeks incubation period. Bernal et al. (2002) added that antagonistic agents with a density of 10^8 cfu ml⁻¹ could colonize the roots but their population in the soil was reduced because of environment. Kumar et al. (2010) added bacteria that are expected to be biocontrol agents must have faster colony growth rather than pathogen growth.

Plant height and wet biomass

The plants that have been inoculated with biofertilizer bacteria tend to had higher growth than those plants without biofertilizer bacteria (Table 1). It showed that bacterial activity in the soil tends to consistently increase plant growth. Application of biofertilizer agents through seed leakage allowed the agent to directly covering the entire root surface so that it could inhibit the entry of FOC pathogen.

The results showed that biofertilizer agents could promote the growth of banana seedlings. Biofertilizer agents are known to produce auxin (Shokri and Emtiazzi 2010). Inhibition mechanism of pathogens by *Azotobacter* and *Azospirillum* through the production of auxin had been able to increase the growth of banana seedlings even though it could not prevent the incidence of wilt disease in the field. Eliza et al. (2007) added that plants could synthesize the auxin hormone from microbes in their tissues to enhancing their growth. Sudarma and Suprpta (2011) added that cv. Ambon Kuning was very susceptible to FOC attacks.

Table 1. Growth and weight of banana plants inoculated with FOC at aged 8 weeks post acclimatization

Treatments (T)	Plant height (cm)	Wet biomass (g)
No biofertilizer (T1)	45.67 ± 02.52 a	41.19 ± 03.11 a
Carrier material (T2)	46.67 ± 03.22 a	37.45 ± 06.69 a
Comparative biofertilizer (T3)	66.67 ± 06.11 b	90.35 ± 10.52 b
<i>Azotobacter</i> (T4)	60.67 ± 09.61 b	59.80 ± 01.23 b
<i>Azospirillum</i> (T5)	61.00 ± 01.00 b	78.20 ± 17.66 b
<i>Streptomyces</i> (T6)	67.33 ± 06.66 b	88.60 ± 10.52 b
<i>Bacillus</i> (T7)	61.33 ± 09.87 b	79.26 ± 02.09 b

Description: The average followed by the same letter is not significantly different based on DMRT at 5%

Observations of the wet biomass on banana seedlings at aged eight weeks after inoculation showed that all treatment had a significant difference. Biofertilizer agents tested both in consortium and independently could trigger the growth of banana plants *in planta*. *Streptomyces* could independently stimulate the cell division to increase the wet biomass. According to Lehr et al. (2008), antagonistic agents had been able to produce auxin, gibberellin, and cytokinin compounds.

The results also showed that banana plants inoculated with biofertilizer agents tended to be more resistant to *Fusarium* attacks than control treatment. Biofertilizer products containing a combination of *Azotobacter*, *Azospirillum*, *Streptomyces*, and *Bacillus* were able to stimulate plant growth. Optimal plant growth could prevent pathogen infections. Cahyani et al. (2014) added that *Fusarium oxysporum* is a weak parasite whereby plants that lack nutrients is prone to pathogen attack.

To conclude, the study showed that biofertilizer agents were able to promote the growth of banana plants. The mechanism of biofertilizers to inhibit FOC is by inducing host plant resistance under greenhouse condition. Several biofertilizer agents had the potential to suppress *Fusarium* wilt *in planta*. *Streptomyces* and *Bacillus* were the most effective in controlling the disease infection. *Azotobacter* and *Azospirillum* had not been able to prevent the plant wilt.

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