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Utilization of Coffee Waste Compost Enriched by Bioactivator to Suppress Basal Stem Rot Disease on Black Pepper

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Abstract

Basal stem rot disease (BSR) has been certainly found in the black pepper plantation in Indonesia. In Lampung, Indonesia, the disease is a major constraint for increasing pepper productivity. This study aims to suppress BSR disease by utilizing coffee waste compost enriched by beneficial bacteria. The beneficialbacteria isolated from the rhizosphere and pepper plant tissues. The bacteria selection based on their ability to dissolve P, K, to fix N, and antagonistic to *Phytophthoracapsici* in vitro. Selection of isolates that the number of bacteria isolates that can be isolated were 187isolates, and 87 isolates of them can be used as an antagonist and dissolved nutrients. Then,only 15 of selected bacterial isolatesweregrouped in 3 formulas based on the great ability as antagonist and nutrient dissolving.

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Each formula consist of 5 isolatesas bioactivatorsenriched in coffee waste compost that to be used in the field test. In the field tests, the utilization of coffee waste compost enriched with beneficial bacteriaformula 2 could suppress BSR disease severity until 97.85%. Plant resistance indicated by increasing of dehydrogenase and peroxidase concentration in plantswere treated by all compost and formulas. The use of coffee waste compost enriched by bioactivator also could improve plant growth in all treatments compared to control.

Keywords: bioactivator; compost; *P. Capsici*; dehydrogenase; peroxidase

1. Introduction

The main limiting factor of black pepper productivity in Indonesia are pests and plant diseases. More than 40,000 ha of black pepper plantation in Lampung, Bangka and West Kalimantan Indonesia infected by *Phytophthora capsici*. This pathogen caused Base Stem Rot (BSR) disease of pepper plant. Stem rot disease causes damage and death of black pepper plants over 40% every year [1].

Recently, there are no effective methods for controlling BSR disease. The reason is the nature of the pathogens that can survive in the soil as saprobes for long time and its attack cause death plant quickly [2]. The use of resistant varieties of pepper plants or fungicides still have many weaknesses. Black pepper plants as perennial crops and virulence variation of *P. capsici* population were attacked pepper, causing high production and black pepper varieties resistant to BSR assambley require a much time [3]. While the use of fungicides will add environmental pollution nd risk. It is necessary to find techniques for effective disease control, compatible, and sustainable in using.

Furthermore, biological research on stem rot disease in black pepper is focused on the identification of the plant rhizosphere microorganisms to suppress the disease, but the utilization of those microorganisms still lack information and application in field. While, Indonesia has a very high microbial diversity, and a lot of them have potential as biological control [4].

Microbes which were as biological agents from bacteria (*Bacillus* spp., *Pseudomonas* spp.), Actinomycetes (*Streptomyces* spp.) and fungi (*Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp.) were the largest microbes present in the compost [5,6]. Some endophytic bacteria such as *P. aeruginosa, P. putida,* and *B. megaterium* has ability to suppress the development of *P. capsici*, which is cause BSR disease on black pepper in India *in vitro* [7]. Combined beneficial bacteria and compost may have great potential to use as formula for decreasing BSR disease on pepper plant.

The research report states that the use of compost can increase the resistance of plants against pathogen attack. Compost is a good substrate for the growth of some biological agent microorganisms such as *Trichoderma* sp., non pathogenis *Fusarium oxysporum* (FoNP) and *Bacillus* sp. Application of compost to the soil can reduce plant pathogen attack [8]. In order to improve the effectiveness of compost needed material such as soil mineral or non-pathogenic microbes.

This study aims were to determine the role of coffee waste compost and bacterial antagonists from the plant tissue and rhizosfir of black pepper to suppress BSR disease.

2. Materials and Methods

2.1 Isolation and Selection of Bacteria for bio-activator on Coffee Waste Compost

Isolation of Bacteria: Isolation of bacteria using a dilution method [9]. Bacteria isolated from the rhizosphere and from roots and leaves tissue of black pepper. Isolation of bacteria from the rhizosphere is done by taking 100 g of soil and added 900 ml of sterile distilled water, shaken using a shaker for 5 minutes. The suspension formed was taken 1 ml and add 9 ml of distilled water in test tube. This work was repeated in order to obtain dilutions ranging 10^{-1} to 10^{-7}. At 10^{-3} up to 10^{-7} dilution of 100 mL was taken and spread on TSA media. Each colony that grew calculated and purified. Isolation of bacteria from leaf tissue is done by grinding 1 g of pepper leaf surface sterilized using 2% NaOCl and washed in sterile distilled water. Sterilization is done by soaking the tissue in a NaOCl solution for 2 minutes and then washed in sterile distilled water while shaken for 2 minutes. Washing was repeated 3 times. The suspension was diluted, start from 10^{-1} to 10^{-5}. Each dilution was taken 100 mL to spread on TSA media. In the same way also to isolate bacteria from the roots. All isolates were derived from tissue be reisolated for purifying, and further characterization is based on the ability to adsorb N, dissolved P and K, as well as antagonism against *P. capsici*. In addition it also tests grams using 3% KOH, hypersensitivity test on tobacco leaves.

Bacteria Selection: Selection to all isolates, both from the rhizosphere or plant tissue based on its effectiveness in dissolving the P and K, adsorb N and antagonistic against *P. capsici*. Effectiveness of dissolve P based on wide clear zone around the colony on Pikovskaya medium. Similarly to P, the ability of bacteria dissolved K element on a selective medium [10], is based on the width of the clear zone around the colony. The ability to adsorb N is based on the formation of ring-shaped white pellicle in semisolid media Nfb. *In vitro* antagonist test was conducted using dual cultere method [11]. Between the candidate of antagonist and *P. capsici* were grown together in PDA medium in Petri dishes. Its capabilities as antagonists determined using the formula: $(R - r) / R \times 100\%$, where R is the radius of the growing *P. capsici* colonies away from antagonist, whereas r is the radius of the advancing toward the antagonist. Selected isolates are used to create a formula that will be used as coffee waste compost bio-activator. There are three bacterial formula, and each formula consists of 5 isolates.

Composting coffee waste: Compost was used in this study is coffee waste. For composting used lignin-degrading bacteria (private collection). Into the ready compost bio-activator bacteria added according to the formula, and then be incubated for 7 days.

2.2 Effect of Coffee Waste Compost Enriched Bio-activator Bacteria Against P. capsici on Infected Plants

Field testing of compost conducted in farmer's orchard, and used a randomized block design. Treatments were use coffe waste compost enriched bio-activator bacteria with 3 replicates, and each replication consisting of 10 plants. The treatment was: a. without a compost as a control, b. enriched compost by bio-activator formula 1, c. enriched compost by bio-activator formula 2, d. enriched compost by bio-activator formula 3. Control treatment only use cow manure. Black pepper plants (± 4 years) which showed BSR treated according to the established in the provision of treatment. In plants that have to be treated with coffee waste compost, each plant was given 1 kg. Sowing compost around the stem. Treatment without compost, the plant was only given 1 kg of cow manure. Consentration of peroxidase and dehydrogenase in plant tissue determined at the end of the reasearch at Indonesian Soil Research Institute, Cimanggu Bogor. Peroxidase measurement used Cohen method [12], while dehidrogenase used modification of Casida method [13].

2.3 Observation

Observation of the growth parameters performed 6 months after treatment include canopy diameter and plant height, while also the severity of disease. To determine disease severity using the formula:

$$KP = \frac{\sum_0^4 (ni \times vi)}{Z \times N} \times 100\ \%$$

KP = disease severity; ni = number of infected plant of certaint catagory; vi = value of numerical catagory; Z = higest numerical value of category; N = Number of plant observed [14].

The development of disease severity BPB of each treatment is determined from 1st month until 6th month after treatment. The development of each disease treatment based on AUDPC values. The calculation of AUDPC following formula [15]:

$$A_K = \sum_{i=1}^{Ni-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

Ak = value of AUDPC on k treatment, y_i = disease severity on ith month, y_{i+1} = disease severity on(i+1)th month, t_i = time when the observation was done on ith month, t_{i+1} = time when the observation was done (i+1)th month. Effectiveness of disease severity suppression is determined by using the formula: $(AUDPC_{control} - AUDPC_{treatment})/AUDPC_{control}$ X 100%

2.4 Data Processing

The results data of bacterial exploration both from the rhizosphere plant tissue just be the number of isolates. All isolates were grouped based on the characterization of dissolving P, K, ability and N fixation, and as an antagonist to *P. capsici*. Disease severity and plant growth (plant high, canopy diameter) were analyzed using a randomized block analysis.

3. Results

3.1 Bacteria from Black Pepper Plant

The result of Bacteria exploration, both from the rhizosphere and plant tissue among healthy pepper plants infected pepper BPB obtained 178 bacterial isolates. The bacterial isolates them have the ability to dissolve existing P, K and N-free absorbing. The percentage of isolates of each of the properties listed in Table 1. Most of Bacterial isolates from the rhizosphere, and they have ability to dissolve K. In the rhizosphere has an organic matter in different types and amounts. Sources of organic matter can be obtained from the root leakage and fertilizers are added. The density of the bacterial population is influenced by the type of plant, plant age, tissue type (roots, stems, and leaves), habitat, and environmental factors.

Table 1. Group of bacteria isolates from plant tissue and rhizosphere of black pepper.

Characteristic	Leaf (%)	Root (%)	Rhizosphere (%)	Total (%)
Dissolved P	11.63	3.49	24.42	36.05
Dissolved K	27.91	8.14	4.65	40.70
Absorb N	6.97	3.49	9.30	16.28

All isolates were tested as an antagonists against *P. capsici*, and it turns out only 87 isolates have the ability as an antagonist. These isolates have the different ability to inhibit *P. capsici in vitro*. Grouping of bacteria isolates ability to inhibit pathogens shown in Figure 1. The results of in vitro testing, the isolates that have a strong ability as an anatagonis no more than 10%. Most isolates that do not have the ability to inhibit *P. capsici* dominated among bacteria that can be isolated.

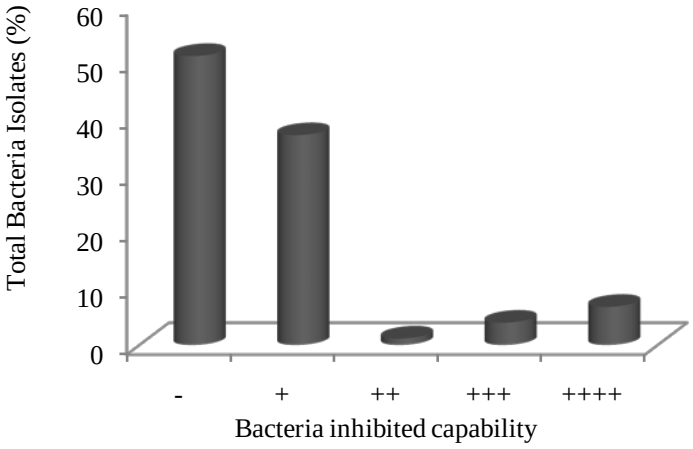

Fig. 1. Bacteria isolates as antagonist to *P. capsicii*. - : no clear zone; + clear zone arround bacteri least than 1 mm, ++ clear zone about 1-2 mm, zona; +++ clear zone between 2-4 mm; ++++ clear zone more than 4 mm.

The results of antagonism ability to inhibited *P. capsici* are known varying diameters. A total of 15 isolates (18.23%) inhibition diameter zone more than 2 mm, 2 isolates (2.29%) 1-2 mm, and 60 isolates of less than 1mm. The number of 18 isolates were isolated from the rhizosphere have capability to dissolve phosphate bond $Ca_3(PO_4)_2$, 13 isolates have the ability to dissolve relatively high in K. Isolation of endophytic bacteria that live inside plant tissues of black pepper were 32 isolates have ability to absorb N from the air.

Among the bacterial isolates showed ability as an antagonist to *P. capsici* was also potentially can improve plant growth. Because it only needs 15 isolates of bacteria, beside as an antagonist there also have properties in help the plant growth, and to induce resistance in plant (Table 2). Bacterial isolates that have the potential to improve the properties of antibiosis and growth is used as a bio-activator in testing in the field, and this is manifested in the form of a bacteria formula.

Table 2. Characteristic of bacteria formula of selected bacteria isolates used as bioactivator on coffee waste compost.

Bacteria isolates	**Characteristic**	**Inhibited (%)**
Formula 1:		
J_4SK_3	Dissolved P and K	48.1 abc
J_1SK_3	Dissolved P and K	54.2 abc
P_1SH_1	Flourescens, dissolved P	68.6 a
L. coffea (2)	Absorb N	52.5 abc
N2SH2	Absorb N	43.2 abc
Formula 2:		
D10	Dissolved P and K	33.8 bcd
N2SK2	Dissolved P and K	42.1 abc
P2SH2	Flourescen, dissolved P	36.7 bcd
P1SH2	Absorb N	22.5 cd
C2	Absorb N	41.4 abc
Formula 3:		
E2	Dissolved P and K	28.8 bcd
$A110^3$	Dissolved P and K	22.5 cd
A105	Flourescen, dissolved P	51.7 abc
H	Absorb N	55.0 ab
H1	Absorb N	45.7 abc

Number with same letter in the same colom no different to Tukey test pada $\alpha = 5\%$.

Testing in vitro treatment of all formulas showed antagonism activity, to be used as the controlling of *P. capsici.* The average diameter of the inhibition zone, the highest are formula 1 and formula 2. Inhibition of bacterial formula to *P. capsici* is different, especialy on formula 3 (table 3). Analysis variance of inhibition zone diameter there are differences in the ability to inhibit the growth formula *P. capsici.*

Table 3. Inhibited zone of bacteria consorsium formula to *P. capsici* on PDA.

Bacteria isolates formula	Average of inhibited zone diameter (mm)
Formula 1	23.16 a
Formula 2	27.27 a
Formula 3	9.00 b

average number followed by the same letter are not different at 5% level Tukey test

Results of identification that has been done on several isolates both from the rhizosphere and plant tissue such as *Pseudomonas* sp., *Bacillus subtilis* and *B. pumilis.* Among these bacteria have the ability to produce antifungal compounds and dissolved nutrients.

3.2 Applications of Bacteria and Coffee Waste Compost on the BSR Disease on Black Pepper

Adding compost and microbial formula can suppress disease BSR 97.23%. The addition of compost to the soil significantly affect the severity of disease caused by *P. capsicii* on pepper plants. All three treatments compost can suppress disease development BPB significantly compared with the control (Table 4). The smallest suppressed occurs in formula 3, and it was consistent with the results of *in vitro* assays against *P. capsici.* Within bacterial isolates formula were given to coffee waste compost does not cause a difference

Table 4. Suppresses of disease severity due to using coffee waste compost enriched bioactivator bacteria on BSR disease on black pepper.

Treatment	Disease severity (%)	Dehidrogenase concent.(µg/ml)	Peroxidase concent. (unit/ mg protein	Dis.suppress (%)
Control, no compost	49.987 a	143.648	0.021	
Compost + formula 1	6.942 b	249.879	0.131	85.36
Compost + formula 2	6.477 b	281.516	0.212	97.65
Compost + formula 3	12.960 b	323.103	0.185	94.12

Numbers followed by the same letter in the same column do not differ by Tukey test α = 0.05. Tests performed after the data were transformed by asinh. Value of disease severity at 6th month after treatment.

Organic matter content of coffee waste compost and microbes in the soil that was given will influence the activity of soil microbes that play a role in the mineralization process, the decomposition of various organic materials and chemical compounds in soil, induced plant resistance. Among the bacterial isolates in formulas derived from plant tissue that has the ability to build up resistance in black pepper. In black pepper that was not given compost indicate the level of *P. capsici* attacks higher and growth rates lower. Coffee waste compost and bacteri in bioactivator very effective suppress BSR disease on black pepper (Table 5).

Tabel 5. Coffee waste compost enrich with bacteria effect to the average width and height and plant canopy, AUDPC and BSR control effectiveness on black pepper.

Treatment	Canopy (cm)	Plant height (cm)	AUDPC	Control effectiveness
Compost + formula 1	88.77 a	139.55 ab	18.67	85,88
Compost + formula 2	83.88 ab	147.89 a	3	97,85
Compost + formula 3	75.22 ab	127.78 ab	7.50	99,61
Control	71.44 b	119.44 b	127.5	

Numbers followed by the same letter in the same column do not differ by Tukey test α = 0.05

4. Discussion

The bacteria were isolated show no same ability in dissolving some nutrient and as an antagonist. To take advantage to suppress *P. capsici* infection, it is necessary to be selected and combined with each other.

The results of these tests indicate that some of the original bacterial isolates and endophytic bacteria produce compounds that classified as antibiotic, beside able to provide the elements that are not available to become available in the soil. Pathogenic fungus would be destroyed enzymatically by β-1.3 glucanases, β -1,4 glucanases and lipases produced by *Pseudomonas fluorescens* Flügge [16]. The antibiotic 2.4 diacetylphloroglucinol (DAPG) produced by *P. fluorescens* is able to inhibit the growth of bacterial leaf blight [17]. The 14 isolates from the rhizosphere of black pepper plants are able to suppress the development of BSR pathogen [8].

One of the effects of biological agents is to stimulate plant growth. Ability increase was due to growth of biological agents that can dissolve P and K was not available in the soil becoming available, produces growth hormone such as indole acetic acid, and produce siderophores [16]. Each formula antagonism test to see with the ability to inhibit the growth of pathogenic BSR (Table 3).

Formula of bioactivator consists of several isolates bacteria. Biologically each formula has a different, so the metabolic activity and secondary metabolism products (such as antibiotics) that produced will different and give different responses to pathogens. Antibiotic compounds produced by antagonistic bacteria may act directly as a bactericidal agent against bacterial pathogens and as inducers (elicitor) plant resistance to disease pathogens. Type of antibiotic products that provide the best response when these antibiotics have the ability to diffuse into the medium and can lead to changes that change the osmotic pressure, surface tension and the changes generally cause damage to the mycelium or changed once completed form will then occur inhibitory action of the enzyme and ultimately metabolism will be hampered. Cyanid acid (HCN) compounds is one of the secondary metabolites produced by *Pseudomonas* spp. are antimicrobial. Antibiotics 2.4 diacetylphloroglucinol (DAPG) produced by *P. fluorescens* is able to inhibit the growth of bacterial leaf blight [17]. Some types of antibiotics produced by *Bacillus* species such as bacitracin, polymyxin, gramicidin, tyrocidine, subtiline, and bacilysin [18].

Treatment compost that enriched with microbe could suppress the levels of stem rot disease on black pepper. Compost increased microorganisms population, bacteria, fungi and actinomycet in black pepper rhizosphere. Microorganisms isolated from soil in the root system of black pepper have potential as biological agents against *P. capsici*. Hendra (2009) reported the results of the use of antagonistic bacteria such as *P. fluorescens* Es32, *P. fluorescens* PG01, and *Bacillus polimyxa* BG25 against *P. capsici* that causing disease BSR disease showed good results in induction of plant growth [19].

Bacteria concortium in formula 2 were used in this experiment have capability to induce plant resistance against BSR disease. It can be seen from the content of peroxidase enzyme produced in plants tested (Table 4). A number of enzymes associated with the induction of systemic resistance, such as peroxidase, phenylalanine ammonia-lyase (PAL), lipoxygenase, β-1.3 glucanase, and chitinase [20]. The high activity of peroxidase associated with cell lignin and papilla formation, as well as the formation of hydrogen peroxide that could inhibit pathogens directly [21]. The increase in peroxidase enzymes and other enzymes that are regulated by the presence of antimicrobial jasmonat acid and ethylene are both activated by saprophytic microorganisms such as rizobakteri [20]. An increase in the content of peroxidase or dehydrogenase in the treatment of waste composting coffee enriched bio-activator as compared with the control associated with the formation of plant resistance (Table 4).

In the wild grapes are experiencing stress due to salinity and powdery mildew and downy mildews work activate genes responsible for the formation of the aldehyde dehydrogenase [22]. Proline dehydrogenase induces a hypersensitive reaction to Pseudomonas syringae infection of Arabidopsis result in a network so that their development is hampered [23]. There will be an accumulation of several enzymes associated with plant defense such as PAL, PPO and POD as a result of pathogen infection on potato soft rot disease [24]. In the black pepper showed resistance against *P. capsici* would show high concentrations of peroxidase and dehydrogenase in tissues.

The use of compost were added to each formula of bioaktivitor not differ from each other in influencing plant growth. (Table 5). Bacteria were added to the compost equally well in supporting the growth and

nutrient supply. The soil with enough organic matter content will form a good soil conditions that help the absorption of nutrients by plants [25]. *Pseudomonas* spp. and *Bacillus* spp. is effective in improving the availability of phosphate in the soil, and it will improve plant growth and yield [26].

Increasing uptake of the elements will strengthening the structure of the black pepper tissue and it prevents the penetration of *P. capsici*. In North Lampung found a low N content and high K can reduce the level of *P. capsici* infection in the field, because the cell walls became hard and thick, carbohydrate and amino acid molecules concentration higher. Availability element is also increasing accordance with the biological activity of microorganisms as well as the population and especially the antagonistic microorganisms. Increased biological activity of microorganisms antagonistic causes *P. capsici* difficult to develop further. This condition is very instrumental in inhibiting further penetration of pathogens in plant tissue [27].

Cause nutrient deficiencies in the control, make plant become weaker. Pathogen easily penetrate the tissue and disrupt physiological processes of plants, especially to the emergence of photosynthetic activity and respiration changes in infected tissues. Changes in photosynthesis and respiration will affect the growth and disease severity will higher. Thee leaves of plants infected with *P. capsici* will be a reduction of fatty acids and not the emergence of photosynthetic activity [28].

5. Conclusion

Based on our research, we success to isolate beneficial bacteria from rhizosphere and tissues of pepper plants with different ability such as in N fixation, P and K solubilization and antagonist of *P. capsici*. Based on result of field test, 15 selected bacterialisolates formulated into 3 formulas as bioactivator enriched in coffee waste compost could suppress development of *P. capsicii*were significant than control. Compared to control, all treatments using these formulas could decrease disease severity, and increase of dehydrogenase and peroxidase content of pepper plants. However, treatment used coffee waste compost enriched by formula 2 causes less disease severity compared to other formulas so this formula can be used in the field for suppressing*P. capsicii* attack in the future.

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