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Research Article

Controlling *Fusarium* damping off on tomato using a bioactive compound enriched by activator microbe and humic acid

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Abstract

Based on results of this research, some concentration of humic acid, 0,1%,0.2% and 0.5% prove that humic acid can promote and inhibit bacteria population growth. In concentration 0.1-0.2% humic acid, activator bacteria can grow very well. However, with concentration 0.5% there are no bacteria can grow in the media. Assessment results of 6 (six) growth media treatments for tomato plants shows that all combination of growth media (compost, activator bacteria and humic acid) have significant effect on suppression of infection by pathogen, *Fusarium oxysporum* with attached level 0-11%, compared with control positive reaching 28% attached level. Combination of growth media can increase plant height respectively 5.81, 6.70 and 6.77 cm compared with control positive of only 5.25 cm. Combination of growth media also can increase root length between 2.82-3.64 cm, compared with control positive of only 2.73 cm. Addition of compost which is enriched by activator bacterium BX2 and humic acid can increase soil microbe populations in growth media of tomato plants thus increasing both soil and plant health.

Keywords: bioactive compost, fungi, activator bacteria, *Fusarium oxysporum*, plant health, Indonesia.

Introduction

Fusarium is one of the important diseases in tomato as it causes a phenomenon known as damping off disease. Intensity of this disease can reach 16,7% in Lembang and Pacet, both important centres for tomato production in West Java, Indonesia [1]. The early symptoms of *Fusarium* damping off are turgor loss and ducked leaves or buds by disruption of the vascular system of roots or stems [2].

Fusarium damping off disease on tomato plants caused by *Fusarium oxysporum f.sp lycopersicum* have characteristics as an active and saprophytic microbe in the soil or as a soil inhabiting fungi. To complete their life cycle, this fungus must pass through most of its life in the host as a parasite of plants and live in tissues of dead plants as a saprophyte in the soil.

Control of *Fusarium* damping off disease is still difficult because this fungus is soil-inhabiting, so it can survive a long time in soil without host plants. The addition of humic acid and organic materials can be used as a source of nutrients for plants to increase the effectiveness of controlling *Fusarium* damping off disease. The addition of humic acids in soil can inhibit the growth of mycelium of *Fusarium culmorum* [3, 4]. This research aims to evaluate the effect of compost enriched by activator microbes and humic acid in controlling the *Fusarium* damping off caused by *Fusarium oxysporum f.sp lycopersici* on tomato plants.

Materials and Methods

Provision of **F. oxysporum** *isolates as pathogen source*

The survey was conducted to determine the land and tomato plants infected with *F. oxysporum*. Tomato plants infected with *Fusarium* disease were used as a source of pathogenic isolates, while the soil is naturally infested by *F. oxysporum* used as planting medium. The survey was conducted in Kampung Pasir China, Cipenewa Village, District Pacet, Cianjur Regency, West Java. Plants suspected of being infected by *F. oxysporum* were cut and washed with 70% alcohol, then washed using aquades for one minute and drained with tissue paper. The plants were then incubated at room temperature for 5-7 days. Soil infected with *F. oxysporum* used as a test planting medium.

Provision of activator bacterial isolates

Pure bacterial isolates were obtained from the Laboratory of Soil Biology and Health, Indonesian Soil Research Institute. The bacterial isolates are B5, B9, B13, B15, BX1 and BX2 (Collection of Surono). The bacteria was tested for its ability to grow on humic acid, to obtain bacteria which can adapt with humic acid and control *F. oxysporum*. Bacteria were cultured on NA and NB media.

Provision of NA media by addition of humic acid

Humic acid was used as a mixture of NA-shaped media solution. The concentration of media made, among others, was 0.1%, 0.2% and 0.5%. Preparation of humic acid solution with a concentration of 10%, *i.e.*, 1 g of finely ground humic acid, dissolved with aquades in the volumetric glass to 10 ml. NA media preparation for the concentrations of humic acid 0.1%, which is concentrated humic acid solution 10% is taken with micrometer pipette 1 ml media and added to NA in the volumetric glass to 100 ml. Making the media 0.2% by adding humic acid solution 2 ml, and added to 100 ml of NA medium. While the media 0.5% by adding of humic acid solution 5 ml, and added to 100 ml of NA medium.

Pathogenicity test of activator bacteria

Bacterial activators have been tested for pathogenicity on tobacco leaves [5, 6]. Pathogenicity testing aims to determine the nature of an organism to cause disease. Bacterial culture injected into tobacco leaves by injection of 1 ml of tobacco leaf. Bacteria that have the nature of pathogenicity will lead to symptoms of necrosis on tobacco leaves. Observations of pathogenicity test were performed 16-24 hours after inoculation.

Growing ability of activator bacteria to humic acid

Tests used NA media, humic acid, B5, B9, B13, B15, BX1 and BX2. The concentration of humic acid used in 0.1% (AS01), 0.2% (AS02), 0.5% (AS05) and control (without humic acid). Each treatment was repeated seven times. Tests using multilevel dilution method [7], up to 10^{-7}. Each treatment was inoculated each bacterial suspension was taken as much as 0.1 ml with a micrometer pipette and dispersed (plating) using a spatula on NA medium (control) and NA humic acid, carried out in laminar flow aseptically. Parameters observed the number of bacterial colonies that grow at 12 hours, 18 hours, 24 hours and 48 hours after inoculation. The population of bacterial colonies is determined by the formula [8]:

$$\text{Population of bacteria} = \frac{x}{p \times v}$$

Description:
x: number of colonies that grow on the plate with the dilution factor (cfu)
p: the dilution factor
v: volume of suspension that was distributed to the cup (ml)

Testing of* Fusarium *attachment and seed vigor of tomatoes

Treatment carried out at the media combination of compost, humic acid, and activator bacteria. Test combinations are listed in Table.1. The test uses bacteria and humic acid concentration of the best in the previous test. Media treatment is the result of mixing between compost and soil infested *F. oxysporum* naturally in the ratio 1:2. The media is inserted in the polybag size of 100 g/polybag. Furthermore, these media were incubated for 1 week. Tests carried out with 5 replications, each replication was repeated ten times, each polybag containing 3 seeds of tomato. Observations were made on 7 days after transplanting (DAT) to 14 DAT with parameters such as incidence of disease, the maximum growth potential (MGP), germination, plant height, number of leaves and root length.

Table 1. Combination of growth media treatments.

No.	Treatments performed
TIAx	Soil without treatment (negative control)
TIAy	Sterilized soil (positive control)
TIA1	Soil + compost
TIA2	Soil + compost + humic acid
TIA3	Soil + compost + activator bacterium
TIA4	Soil + compost + activator bacterium + humic acid

Description: TIA: Naturally infested soil

Analysis of microbial populations of growing media

Analysis was performed on soil microbial population before planting and after treatment. Each soil sample was taken as much as 10 g, then added with a solution of NaCl (8.5 g / 1litre) of 90 ml included in Erlemenyer. Soil suspension was homogenized with shaker, 150 rpm for 30 minutes. Dilution made up to 10^{-6}. At the dilution 10^{-4}, 0.1 ml was taken with micrometer pipette, plated on PDA medium with a spatula. This work was repeated three times and incubated at room temperature for 5-7 days. 10^{-6} dilution was taken as much as 0.1 ml, grown on NA medium with three replications and incubated at room temperature for 5-7 days. Observation colonies such as the number and diversity of microbial populations. Isolation was carried out aseptically in laminar flow. Microbial colony population is determined by the formula:

$$\text{Population total} = \frac{\text{Number of microbe colonies which grow with the dilution factor- (cfu)}}{\text{Dilution factor to- x spreaded volume (g)}}$$

Experimental design

This research used a completely randomized design (CRD). Observation data were analyzed by using Microsoft Office Excel 2007 and SAS 6:12, Duncan's multiple range test with significance level α = 5%.

Results and Discussion

Preliminary test

Tests in the laboratory showed that in the pathogen-infected tomato plants appear white mycelium around 5-6 days after incubation, was followed by identification of mycelium colonies by making preparations. The observation of preparations with phase contrast microscope shows the morphology of fungi conidial form are round, length, and tapered like a rounded crescent [9]. The morphology of these forms has conformance with *F. oxyporum* so the soil of tomato plants have been naturally infested with *F. oxysporum*.

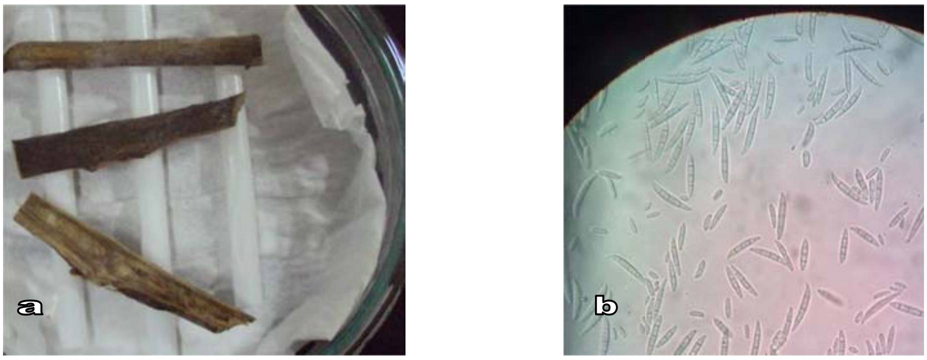

Figure1. *F. oxyporum* mycelium arising in symptomatic plants (a) and observed under the microscope (b).

The soil-borne pathogen*, Fusarium oxysporum* is one of the common diseases causing *Fusarium* wilt in crops of *Solanaceae*: tomato, potato, eggplant and chili. This disease causes serious seedling damping-off. *Fusarium* also causes plants to grow abnormally, or uses the plant as an agent for the pathogen transmission to other host plants. The pathogen infects young roots, growing, developing and spreading in roots and stem vessels, inhibiting water and nutrient transport [10, 11]. Some members of *Fusarium* are aggressive pathogens that cause either vascular wilt or root and stem decay [12]. Pathogenic isolates have traditionally been identified by their ability to elicit plant disease symptoms [13].

Pathogenicity test of activator bacteria

Based on pathogenicity tests have been done (Table. 2), showed that all bacteria used in this study none of which produces symptoms of necrosis on tobacco leaves. Thus, bacterial activators used in this study did not have the ability to cause disease to plants. So, all bacteria are non-pathogenic bacteria.

Table 2. Pathogenicity test of activator bacteria in tobacco leaves.

No.	Bacteria	Pathogenecity
1	B5	-
2	B9	-
3	B13	-
4	B15	-
5	BX_1	-
6	BX_2	-

Description: (+): Potential pathogenicity, (-): No cause pathogenicity

Ability of activator bacteria to grow in humic acid

Results of analysis of the influence of NA medium with the addition of humic acid showed a significant effect on the formation of activator bacterial colonies. The addition of humic acid generally increases the population of activator bacteria, except in BX1 (Table 3). This is due to organic materials, such as humic acid, as a source of energy for the macro-and micro-organisms in the soil [14], so that the total of activator bacterial population increases. Increasing number of activator bacterial populations to the addition of humic acid of 0 to 35.43%.

Table 3. Effect to bacterial population growth on NA medium with the addition of humic acid.

Bacteria	Control	**Bacterial population in treatment** (10^7 cfu/ml)*		
		AS01	AS02	AS05
B5	2,333a	9,857a	4,714a	0,143a
B9	27,667a	26,714a	0,429b	0b
B13	2,333ab	4,571a	2,000ab	0b
B15	8,333ab	10,143a	3,714bc	0,429c
BX_1	40,00a	8,57b	2,14b	0b
BX_2	10,333b	16,000a	0,143b	0c

* The average same row followed by different letters indicate results significantly different (Duncan test interval α = 0.05)

Testing of* Fusarium *attack and seed vigor of tomatoes

The results showed that the addition of humic acid and activator bacterium BX2 influenced the incidence of disease, the potential for maximum growth, germination, leaf number, plant height and root length of tomato (Table.4). Effect of humic acid and bacterium BX2 interaction occurs in all observed variables (Table 4).

The addition of humic acid and activator bacterium BX2 inhibits the damping off from 62.38 to 69.52% (Table 4). Addition of humic acid and BX2 is able to increase the potential for compost and soil microbial populations to inhibit the disease incidence of *Fusarium* damping off. This is supported by data analysis of microbial populations, that an increasing number of population of bacteria, fungi, and actinomycetes cause nutrient competition with pathogens causing damping off in the planting media. According to Soesanto [15], the diversity of microbial populations is one factor of inhibiting damping off disease, competition in the soil nutrient sources utilized by soil microbes to increase the number of colonies and diversity, thereby reducing the total population or the effectiveness of soil borne pathogens.

Table 4. Effect of addition of humic acid and bacterium BX2 in the tomato growing medium.

Treatment	Parameter*					
	Incidence of disease (%)	**Germination** (%)	**MGP**** (%)	**Plant height** (cm)	**Number of leaves** (leave/plant)	**Root length** (cm)
TIAx (Control (-))	28,02a	73,2c	78,62c	5,2589c	10,46c	2,730c
TIAy (Control (+))	0c	89,36a	93,14a	6,337a	17,82a	2,822c
TIA1	10,54b	81,8ab	87,88ab	6,4024a	15,12b	3,220abc
TIA2	8,54b	81,28ab	89,94ab	5,8122b	14,3b	2,940bc
TIA3	11,14b	83,2ab	88ab	6,7056a	14,28b	3,440ab
TIA4	10,54b	78,02bc	85,14b	6,772a	14,08b	3,640a

* The average in same lane followed by different letters indicate results significantly different (Duncan test interval $\alpha = 0.05$)

** Maximum growth potential

The addition of humic acid and activator bacterium BX2 on compost media can increase the potential for seed germination is normally higher than the negative control. In addition, the potential for growing seed increased by 7.66 to 12.52%. This is due to the addition of humic acid increases the release of nutrients in the soil [16], so that the seeds of plants absorb more nutrients and grow better. Humic substances are the most abundant form of organic matter in terrestrial ecosystems and microbial interactions with these materials may be fundamental in many ways [17, 18]. Many beneficial effects of humic substances on the growth of plants are recognized as they can produce various morphological, physiological and biochemical effects on plants [19, 20].

The addition of humic acid and activator bacterium BX2 can also increase the growth of plants (Table 4). This is because the growth media a given organic matter and activator microbes become a better growing medium. Harja and Sudirman [21], stated that addition of humic acid in soil had significant affects on plant height and biomass of legumes.

Based on the observations (Table 4), the addition of humic acid and activator bacterium BX2 provides the ability of seeds to germinate is higher, more number of leaves and root length increased, respectively increased by 6.18 to 12.01%, 25.71 - 30.82%, and 21-25%. This shows the addition of organic material will improve the ability to hold and absorb water so the ability to provide ground water to rise as a result of plant growth increased growth. Generally, addition of humic substances jointly with inorganic fertilizer improves the soil fertility status at different soil layers [22].

Many soils and especially rhizosphere bacteria can stimulate growth of crops, in the absence of a major pathogen, by directly affecting plant metabolism. These bacteria belong to diverse genera, including *Acetobacter, Achromobacter, Anabaena, Arthrobacter, Azoarcos, Azospirillum, Azotobacter, Bacillus, Burkholderia, Clostridium, Enterobacter, Flavobacterium, Frankia, Hydrogenophaga, Kluyvera, Microcoleus, Phyllobacterium, Pseudomonas, Serratia, Staphylococcus, Streptomyces* and *Vibrio*, as well as the well-known legume symbiont *Rhizobium* [23]. Rhizosphere bacteria are present in large numbers on the root surface, where nutrients are provided by plant exudates and lysates. Certain strains of rhizosphere bacteria are referred to as plant growth–promoting rhizobacteria (PGPR), because their application can stimulate growth and improve plant stand under stressful conditions [24].

Analysis of microbial populations in the growing media

Results of soil biology analysis showed that the addition of humic acid and BX2 increases the population of bacteria, fungi and actinomycetes, respectively increased by 39.93 to 99.13%, 79.69 to 97.96% and 0 - 100%. According to Wongso [14], states that the addition of organic matter can increase populations of soil microorganisms, including fungi and actinomycetes. Improvement of population of colonies of bacteria, fungi, and actinomycetes may decrease the incidence of disease on tomato seedling collapse. Table 5 presents data analysis results and the diversity of microbial populations of growing media before and after the addition of humic acid and activator bacterium BX2.

Table 5. Microbial populations and diversity of planting medium before and after the addition of humic acid and activator bacterium BX2.

Treatment		Population (cfu/g)		
		Bacteria	Fungi	Actinomycetes
Before	TIAx (Control (-))	0 -2 x 10^5	0-0,67 x 10^5	-
	TIA1	0-1,1 x 10^6	0-3,3 x 10^6	0-6,7 x 10^6
After	TIAx (Control (-))	0-3,33 x 10^5	-	0-3,3 x 10^6
	TIAy (Control (+))	0-1,5 x 10^6	-	-
	TIA1	0-3,67 x 10^5	0-3,3 x 10^6	0-1,67 x 10^5
	TIA2	0-3,33 x 10^5	-	0-1 x 10^5
	TIA3	0-1,5 x 10^6	0-1 x 10^5	0-6,7 x 10^6
	TIA4	1,567 x 10^6 - 2,3 x 10^7	0-3,3 x 10^6	-

Conclusion

Compost enriched by humic acid and activator bacterium BX2 give positive effect on the improvement in germination to inhibit infection of *Fusarium* sp. causing damping off in tomato plants. Activator bacterium BX2 is a bacteria which increases the potential of organic matter in controlling damping off and improves agronomic potential of tomato plants. Addition of humic acid with concentration of 0.1% and activator bacterium BX2 can suppress *Fusarium* damping off disease from 62.38% to 69.52%.

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