Tomato Plant Protection Management to Reduce Fusarium Wilt Disease by Utilizing Plant Growth Promothing Rizobacteria *Pseudomonas alcaligenes*

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Abstract- Tomato plants are susceptible to Fusarium wilt disease with a field attack rate of 10 to 20%. The research was conducted to get a simple and can be done by farmers by using Plant Growth Promothing Rizobacteria (PGPR). Research was conducted in the laboratory to determine the biochemical characteristics of rizobacteria and in the greenhouse to determine the effect on plant growth. Tests conducted on three isolates of bacteria P. alcaligenes which is the result of previous research. The results showed that the rhizobacteria tested were gram negative bacteria, did not produce enzyme oxidase, produced catalase enzyme, glucose fermentation, lactose and sucrose, did not produce gas and cyanide acid. The three rizobacterial isolates are able to spur the growth of tomato plants through soaking the seeds with PGPR suspense for 20-30 minutes. Application of P. alcaligenes isolates has been shown to increase total phenol content up to 262% and 606% salicylic acid in tomato plant tissue. Soaking tomato seeds with P. alcaligenessuspense suppressed the tomato wilt disease 44.44 - 55.56% and were able to increase the yield of 3 to 4 times more than the control plants.

Keyword: PGPR, P. alcaligenes, biochemical characteristics, Fusarium wilt, soaking of seeds

I. Introduction

Fusarium wilt disease in tomato plants caused by the fungus *Fusariumoxysporum*f. sp*lycopersici*, is an important disease in tomato plants and have resulted in massive damage to various tomato-producing countries in the world (Rosewich*et al*, 1999). In Indonesia, the disease was recorded percentage reached 16.7% in Lembang - West Java and 10.25% in Malang - East Java (Semangun, 2007). Fusarum wilt disease can cause death in tomato seedlings as soon as the first symptoms appear, while in adult plants, the plants will wither and die suddenly when in severe infections and weather favorable for pathogens (Agrios, 2005). Control of *Fusarium* wilt disease has not worked well because the *Fusarium* is soil borneand can survive for a long time in the soil without host plants, so the crop rotation becomes inffective. Controlling the disease by applying synthetic fungicides into the soil can only suppress *Fusarium* wilt for a few months (Alabouvette *et al*., 1996), in addition the use of synthetic fungicides continuously can also cause more resistant pathogens and will contaminate environment (Freeman *et al*., 2002)

Various attempts have been made in controlling *Fusarium* wilt disease, like the use of healthy seed, crop rotation, intercropping and use of pesticides (fungicides), but has not given satisfactory results. Another alternative for controlling *Fusarium* wilt disease and being reported effectively utilization of microbial biological control agents (Freeman *et al*., 2002). Microbes that are beneficial to plants, such as rizobacteria from *Pseudomonas* group with function can enrich the soil, that are important for biological control of plant pathogens and can increase plant resistance (*induced systemic resistance* ISR) (McMilan, 2007). Rizobacteria works directly as a biological fertilizer and biological stimulant by producing plant growth hormones such as IAA (*indole acetic acid*), *gibberelin, cytokinin, ethylene,* dissolved minerals, and also indirectly prevents pathogenic microorganisms through figuration of siderophore and antibiotic (McMilan 2007 ; Sarma *et al*., 2009). This research is to investigate the mechanism of rizobacteria action in reducing the percentage of Fusarium wilt on tomato plants, to determine the ability of *P. alcaligenes* rizobacteria suppress Fusarium wilt disease and to spur growth and increase the yield of tomato plants.

II. METHOD

Location and Period of Research

In vitro research were held in the Laboratory of Agriculture Faculty,Mahasaraswati University of Denpasar In vivo research were held in greenhouses which is located in Denpasar Bali. The study or researchment began in Juni and September 2016.

Isolation of *F. oxysporum*f.sp. *lycopersici*

Isolate of *F. oxysporum f.sp. lycopersici* is obtained from the stems of tomato plants wilt disease. The plant material has previously been disinfected with 70% alcohol and rinsed with sterile water, then cut it into pieces with a size ± 0.5 cm and plant it on PDA that were placed in a petridish. Purification is occured if there are other microbes that grow along the target fungi. Incubation was performed at room temperature, in 3 days pathogenic fungi spores would grow and could be used as a source of inoculum. To ensure that the fungus is *Fusarium* wilt pathogens so making a test by following the procedure of Koch's postulates is needed.

Re-Isolation ofrizobacteria *P. alcaligenes*

*P. alcaligenes*isolates used in this study is the result of previous research that demonstrates the ability antagonistic to the pathogen Fusarium. *P. alcaligenes* used in this study consisted of 3 (three) isolates namely KtS1, TrN2 and TmA1 (widnyana *et al*., 2013)

Testing the characteristic of biochemistryisolatrizobacteria*P. alcaligenes*

Test biochemical characteristics rizobakteri carried out by 13 types of tests include Gram stain, Oxidase, Catalase, Triple Sugar Iron Agar (TSIA) (including the ability to synthesize glucose, lactose, and fructose), Gas, H2S, Indol, motile, MR (metal red), VP (VogesProskuer), and KB (King, SB). Identification of rhizobacterial isolates begins with Gram staining because this technique is one of the first steps to identify the bacterial cell that separates bacteria into 2 groups: Gram positive (purple / blue) and Gram negative (red).

Testing the potency of plant resistance through phenol and salicylic acid content of tomato plants

Analysis of the total of phenol and salicylic acid conducted using HPLC (*High Performance Liquid Chromatografi*). On every analysis of tomato plants use 3 units of tomato plants with part of stem and leaves. Then the part of those are combined into a single sample prepared for analysis by weight of each sample 50g part of a plant.

The ability test to biocontrol agent and as a PGPR on tomato plants

Research using Randomized Block Design (RBD) with Factorial pattern. The first factor is the type of *P. alcaligenes* isolate and the controls consist of four types, they are: K (sterile water), KtS1 (isolate 1), TrN2 (isolate 2), and TmA1 (isolate 3), the second factor is the treatment manner of applying *P. alcaligenes* isolate bacterial suspension. ie by floating roots of tomato seedlings (A), tomato seed soaking (B), and nursery watering tomato seedlings (C). So that there are 12 combinations of treatment, they are: KA, KB, KC, KtS1A, KtS1B, KtS1C, TrN2A, TrN2B, TrN2C, TmA1A, TmA1B, TmA1C. Each treatment was repeated 3 times, so there are 36 experimental units, and each unit consists of 3 plants experimental trials. Total plant in the experiment is 12 x 3 x 3 = 108 experimental plants.

Application of isolate of *P. alcaligenes* bacteria through tomato seeds soaking for 30 minutes and then seeded first on moist cotton layer and a thin cotton layer covered for 5 days (until sprout appeared), then transferred to medium soil and sand (with 1:1 ratio) which is sterile. Floating roots of tomato seedlings performed for 10 minutes at old seedlings 14 days after sowing. Applications are made through watering tomato seedlings in the nursery 10 days old. Inoculation of *F.oxysporum* f.sp *lycopersici* spore suspension was done by spraying 20 ml of spore suspension three days before the tomato seedlings are planted in polybags.Parameter which is observed are: percentage wilt diseases, plant height and the amount of leaves are observed , the fruits weight, the fruits amount, the weight of unit tomato is counted after the plants on X week .

III. RESULT RESEARCH

Biochemical activity of *P. alcaligenes*

Biochemical tests conducted at the Maros Laboratory of South Sulawesi aim to obtain information on the biochemical activity of rizobacteria, the results are as presented in Table 1.

Bacterial isolates	Gram staining			TSIA				OF
		Oxidase	Catalase	Butt	Slant	Gas	H2S	
P. alcaligenes KtS1	-	-	+	Y	Y	-	-	A
P. alcaligenes TrN2	-	-	+	Y	Y	-	-	A
P.alcaligenes TmA1	-	-	+	Y	Y	-	-	A

Bacterial isolates	SIM					IMViC			
	indol		motil	Gas	H2S	MR	VP	KA	KB
P. alcaligenes KtS1	-		+	-	-	-	-	+	+
P. alcaligenes TrN2	-		-	-	-	-	-	+	+
P.alcaligenes TmA1	-		-	-	-	-	-	-	+

Description:

Y = Yellow, A = alkaline,
-(negative reaction),
+(Positive reaction)

Table 1.show that the bacteria *P. alcaligenes* KtS1, *P. alcaligenes* TrN2, *P. alcaligenes* TmA1 have similarities in 13 parameters of biochemical activity such as gram negative bacteria, not producing enzyme oxidase, producing catalase enzyme, fermentation glucose, lactose and / or sucrose, does not produce gas and cyanide acid.

Biochemical tests commonly used in bacterial identification activities include TSIA (Triple Sugar Iron

Agar), Voges-Proskauer test, Red Methyl test, Indol test and others. Biochemical testing is one of the most important things in the world of microbiology (Lim, 1998). TSIA tests are designed to distinguish some types of bacteria belonging to the Enterobacteriaceae group, which are gram negative and ferment the glucose to form an acid so that it can be distinguished from other intestinal gram-negative bacteria. Indol test is used to test the conversion of tryptophan to metabolite product and to oxidize by bacteria with enzymatic activity. the ability to hydrolyze tryptophan by producing indole is not a characteristic of all microorganisms, thus meaning can be used as a biochemical marker.

Bacteria have various biochemical activities by using nutrients obtained from the surrounding environment. Biochemical transformations can occur inside and outside of the bacteria is regulated by enzymes. Each bacterium has the ability to use its enzyme for the degradation of carbohydrates, fats, proteins, and amino acids. This metabolism usually produces products that can be used for bacterial identification and characterization. In principle, observations of biochemical activity or metabolism of microorganisms are known from the ability of microorganisms to use and decompose complex molecules and simple molecules. In addition, metabolism often yields byproducts that can be used for identification (Ramaisyah, 2011).

Metabolic activity is inseparable from the presence of enzymes. Based on the workplace, the bacteria have two types of enzymes namely endoenzyme and eksoenzim. Endoenzymes are enzymes that work in cells. Endoenzyme systems other than anabolic can also be catabolic, while exoenzim is an enzyme that is secreted out of the cell and diffuses into the media. Most of the ecsoenzymes are hydraulic, which means that eco-polymers decompose complex molecules into molecules that are simpler molecules. These smaller molecules can then enter the cell and be used for the benefit of the cell (Waluyo, 2004).

Effect of *P.alcaligenes*isolates Treatment on Plant Height, Number of Leaves and Percentage of Tomato Fusarium Wilt Disease

The results of statistical analysis showed that treatment of bacterial isolates of *P.alcaligenes* and how the application was highly significant (P <0.01) to the height, leaf number and percentage of wilt disease of tomato plants. There is a significant interaction (P <0.05) between the treatment of bacterial isolates of *P.alcaligenes* by means of its application to plant height and number of leaves on the observation week X. There is no significant interaction between treatment types of bacterial isolates *P.alcaligenes* by means of the application of the percentage of *Fusarium* wilt disease. At week X only treatment that isolates a very significant impact (Table 2).

Table 2. Effect of *P.alcaligenes* isolates Treatment on height, number of leaves, andthe percentage of tomato fusarium wilt disease

Isolates	Application	Plant height(cm)	Number of leaves	Desease percentage
Control	A. Floating root	36,10 d	78,56 f	100 a
	A. Soaking seed	36,10 d	78,56 f	100 a
	A. Watering seedling	36,10 d	78,56 f	100 a
KtS1	A. Floating root	87,71 c	109,22 e	66,67 ab
	A. Soaking seed	114,12 ab	167,56 b	55,56 b
	A. Watering seedling	98,47 c	150,11 bc	55,56 b
TrN2	A. Floating root	97,60 c	118,44 de	66,67 ab
	A. Soaking seed	116,30 a	182,22 a	55,56 b
	A. Watering seedling	104,48 bc	149,78 bc	55,56 b
TmA1	A. Floating root	98,25 c	129,44 cd	55,56 b
	A. Soaking seed	120,44 a	192,11 a	44,44 b
	A. Watering seedling	105,54 bc	157,67 b	44,44 b

Description: The numbersfollowedbythe same letterin the same columnarenot significantdifferentat5% DMRT

Effect of *P.alcaligenes* isolates Treatment on the total Phenol and Salicylic Acid Content on Tomato Plants

The data in Table 3. shows that the increase in total phenol content of the highest in the three types of bacterial isolates *P.alcaligenes* (TmA1, TrN2, and KtS1) occurred in the treatment of bacterial application through seed soaking (B), followed by treatment with watering nursery seedlings (C) and floating root (A).

Table 3.The content of total phenols and salicylic acid tomato plant tissue after of *P. alcaligenes* isolates treatment

The treatment of bacterial isolates	Total phenol (%)	Salycilid acid (%)
Control	0,26	0,17
*P. alcaligenes*KtS1 A	0,33	0,23
*P. alcaligenes*KtS1 B	0,52	1,03
*P. alcaligenes*KtS1 C	0,50	0,27
*P. alcaligenes*TrN2 A	0,39	0,29
*P. alcaligenes*TrN2 B	0,63	1,10
*P. alcaligenes*TrN2 C	0,57	0,37
*P. alcaligenes*TmA1 A	0,29	0,24
*P. alcaligenes*TmA1 B	0,94	1,20
*P. alcaligenes*TmA1 C	0,49	0,52

Description: A = floating the roots, B=seed soaking , C = watering nursery seedlings

Regression analysis between the percentage of salicylic acid content of *Fusarium* wilt disease demonstrate the value of R2 = 0.47, with the regression equation Y = 74.024 to 63.82 X, where X is the salicylic acid content in tissues of tomato plants. This means that the increase in resistance of tomato plants against *Fusarium* wilt disease is indicated by an increase in salicylic acid content in tomato plant tissue, which is one indicator of plant resistance to pathogens. This is in accordance with the opinion of De Meyer and Hofte (1997), and Maurhofer*et al.* (1998) which states that salicylic acid is a metabolite of plants in response to *Pseudomonas* indicated spur increased plant resistance.

Effect of *P.alcaligenes* isolates Treatment on production of tomato plants

The treatment of bacterial isolates of *P. alcaligenes* (TmA1, TrN2, KtS1) gives a significant influence (P<0.01) to total fruit, total fruit weight and weight per fruit of tomato. There is a highly significant interaction effect (P<0.01), especially against the total tomato fruit weight parameters. Results of statistical analysis of treatment effects to yield components of tomato plants that fruit number, total fruit weight, and weight per fruit of tomato are presented in Table 4. Occurs very significant interaction effect (P<0.01) between the treatment of bacterial isolates and how its application to the total weight of the fruit.

Table 4. Effect of *P.alcaligenes* isolates treatment on production of tomato plants

Isolate	Application	Number of fruits	Weight per plant (g)	weight per fruit (g)
Control	A. Floating root	30,56 c	84,00 e	2,83d
	B. Soaking seed	30,56 c	84,00 e	2,83d
	C. Watering seedling	30,56 c	84,00 e	2,83d
KtS1	A. Floating root	41,89 b	231,56 e	5,11bc
	B. Soaking seed	55,00 a	278,33 d	5,13bc
	C. Watering seedling	48,78 b	241,00 d	5,00bc
TrN2	A. Floating root	58,78 a	237,22 d	4,05cd
	B. Soaking seed	70,67 a	393,06 b	5,88ab
	C. Watering seedling	62,89 a	330,44 c	5,34bc
TmA1	A. Floating root	54,89 b	259,44 d	4,80bc
	B. Soaking seed	64,33 a	451,89 a	7,15a
	C. Watering seedling	58,89 a	376,33 b	6,65ab

Description: The numbersfollowedbythe same letterin the same columnarenot significantdifferentat5% DMRT

The treatment with bacterial isolates *P.alcaligenes* TmA1 by way of seed soaking application (B) is a treatment that is able to give effect to the growth of the plant is the highest number of leaves (192.11 strands) , the highest tomato plant height (120.44 cm) , and production is the total weight of the heaviest tomato fruit (451.89 g) , and gives the highest resistance to *Fusarium* wilt disease in which up to a week IX no affected plants . The above is followed by treatment TrN2B the number of strands of leaves reaching 182.22, reaching 116.30 cm plant height , and total fruit weight totaled 393.06 g/tree , with the percentage of disease only reaches 11 % at week IX . Against the total fruit weight of tomato , treatment TmA1B gives the highest total fruit weight 451.89 g/tree , followed by TrN2B with a total weight of 393.06 g/tree , and TmA1C is 376.33 g/tree , while at the controls 84.00 g/tree . Plant growth and the production of the best on TmA1B treatment occurs as a result of competition rizobacteria*P. alcaligenes*with pathogens is to form a siderophore under conditions of maximum and induction of resistance by rizobacteria indicated by an increase in the content of total phenols and salicylic acid are the highest compared to other treatment.The results of the regression analysis between the percentage of *Fusarium* wilt disease to the total weight of tomato fruit demonstrate the value of R2 = 0.899 with the regression equation Y = 391.854 to 3.21 X, where Y is the total weight of tomatoes and X is the percentage of wilt disease. This shows that the higher the wilt disease percentage, the total weight will increasingly are reduced.

The results of the study suggests that the isolates were capable of functioning as a plant growth promoter (Plant Growth PromothingRizobacteriaa/PGPR) and also functions as an Induced systemic resistance/ISR is a bacterial isolates of *P. alcaligenes*TmA1B, followed by TrN2B, and TmA1C. The best way that isolates the application is through seed soaking for 20-30 minutes, and by watering in the nursery seedlings of tomato plants is a second great way.

Tomato Agribusiness Business Analysis (conventional)

a. Assumption

1. Land area of 10,000 m² with rental system Rp700.000 per month.
2. Period of calculation of business analysis done every six months.
3. Tomato yields are divided into two grades, namely A-B grade of 30,000 kg with selling price Rp2.000 / kg, and grade C as much as 20,000 kg with the selling price of Rp1.000 / kg.

b. Cost calculation

- Investment Cost

Component	Unit	Price (Rp)	Amounth (Rp)
Agriculture Tools	4 set	200.000	800.000
Plastic Bucket	10 pcs	20.000	200.000
Scales	2 pcs	80.000	160.000
Crop Box	8 pcs	100.000	800.000
Gembor	8 pcs	75.000	600.000
Sprayer	2 pcs	350.000	700.000
Total BiayaInvestasi			**3.260.000**

— Fixed Cost

Deccription	Life time	Price (Rp)	Depreciation (Rp)	Total Cost (Rp)
Rent of Land 5.000 m²	6 bulan	700.000		4.200.000
Depreciation of Agriculture Tools	36 bulan	800.000	4/36 x 800.000	133.333
Depreciation of Plastic Bucket	24 bulan	100.000	4/24 x 200.000	50.000
Depreciation of Scales	36 bulan	160.000	4/36 x 160.000	26.667
Depreciation of Crop Box	36 bulan	500.000	4/36 x 800.000	133.333
Depreciation of Gembor	24 bulan	375.000	4/24 x 600.000	150.000
Depreciation of sprayer	60 bulan	350.000	4/60 x 700.000	70.000
Total Fixed Cost				**4.763.333**

— Variabel Cost

Uraian	Satuan	Harga (Rp)	Total Biaya (Rp)
Seed	12 wrap	75.000	900.000
Manure	10.000 kg	300	3.000.000
Urea fertilizer	300 kg	1.400	420.000
Fertilizer SP-36	300 kg	1.900	570.000
FertilizerZA	200 kg	1.200	240.000
PupukKCl	250 kg	1.800	450.000
NPK pearl subsequent	400 kg	8.000	3.200.000

fertilizer			
Lime of agriculture	2.000 kg	300	600.000
Insecticide	25 liter	150.000	3.750.000
Fungicide	25 kg	70.000	1.750.000
String of raffia	15 roller	5.000	75.000
Stake	20.000 stem	150	3.000.000
Clothesplastic	75 meter	3.000	225.000
Polibag	15 kg	30.000	450.000
Mulsa plastic	11 roller	350.000	3.850.000
Nursery workers	60 HKW	15.000	900.000
Labor processing land	175 HKP	20.000	3.500.000
Manpower of planting	50 HKW	15.000	750.000
Maintenance workforce	250 HKP	20.000	5.000.000
Maintenance workforce	200 HKW	15.000	3.000.000
Harvest and post harvest labor	50 HKP	20.000	1.000.000
Harvest and post harvest labor	100 HKW	15.000	1.500.000
Total BiayaTidakTetap			**38.130.000**

Description: HKW = Working Day Woman (6 hours a day)
HKP = Day Worker Man (8 hours a day)
- Total Cost of Operations per Period
Total operational cost = Total cost / total cost variable
= Rp4.763.333 + Rp38.130.000
= Rp42.893.333
c. Advantages and Advantages
- Revenue per Period
Revenue = Number of sellers x price of Tomato
Revenue of grade A-B = 30,000 kg x Rp2.000 / kg = Rp60,000,000
Income from grade C = 20,000 kg x Rp1.000 / kg = Rp20.000.000
Total Revenue = Rp80,000,000
- Profits per Period
Profit = Revenue - Total operational cost
= $ 80,000 - $ 42,893,333
= Rp37.106.667
d. Feasibility
- R / C Ratio
Ratio R / C = Revenue: Total operating costs
= $ 80,000: Rp42.893.333
= 1.87
R / C is more than the other one is a business and is not operable. R / C 1.87arts of each additional capital of Rp1 will provide a revenue of Rp1.87.
- Pay Back Period
Pay back period = (Total cost of investment: profit) x 1 month
= (Rp3.260.000: Rp37.106.667) x 1 month
= 0.09 months
That is, the turnaround of capital tomato cultivation can be achieved is not preferred (0.09 months).

Tomato Agrobusiness Business Analysis (by using seeding seeds with TmA1rizobacteria)
Financing with details above
Total yield per hectare (Soaking seeds TmA1) = 58,250,000kg

Advantages and Advantages

- Revenue per Period
Revenue = Number of sellers x price of tomato
Revenue of grade A-B = 35,000 kg x Rp2.000 / kg = Rp70,000,000

Income from grade C = 23,250 kg x Rp1.000 / kg = Rp23.250.000

Total Revenue = Rp 93.250.000
- Profits per Period
Profit = Revenue - Total operational cost
= Rp 93.250.000 - Rp42.893.333
= Rp 50,357,000
d. Feasibility
- R / C Ratio
Ratio R / C = Revenue: Total operating costs
= Rp93.250.000: Rp42.893.333
= 2.17
- Pay Back Period
Pay back period = (Total cost of investment: profit) x 1 month
= (Rp3.260.000: Rp50.357.000) x 1 month
= 0.09 months

That is, the turnover of capital tomato cultivation can be achieved is not preferred less one mount (0.06 bulan).
Table: production of tomatoes during harvest at each treatment

Isolate	Treatment	Production at first harvest per plant (g)	Total production during harvest (g) per plants	Total weight per ha (25000 plants) (g)
Control	A.Floating root	84	470	11,750,000
	B.Soaking seed	84	483	12,075,000
	C.Watering seedling	84	500	12,500,000
KtS1	A.Floating root	231.56	1390	34,750,000
	B.Soaking seed	278.33	1660	41,500,000
	C.Watering seedling	241	1446	36,150,000
TrN2	A.Floating root	237.22	1420	35,500,000
	B.Soaking seed	393.06	2150	53,750,000
	C.Watering seedling	330.44	1980	49,500,000
TmA1	A.Floating root	259.44	1556	38,900,000
	B.Soaking seed	451.89	2330	58,250,000
	C.Watering seedling	376.33	2098	52,450,000

IV. CONCLUSION AND SUGESSTIONS

1. Isolates of *P. alcaligenes*KtS1, TrN2, and TmA1 effectively reduce the percentage of *Fusarium* wilt on tomato plants in a greenhouse experiment.
2. The treatment with *P.alcaligenes* TmA1 isolates applied by soaking the seeds of tomato (TmA1B) is

the most effective treatment in reducing the percentage of *Fusarium* wilt on tomato plants.

3. Application of *P. alcaligenes* isolates has been shown to increase the total content of phenol and salicylic acid in tomato plant tissues

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