Effectiveness of microencapsulant *Bacillus subtilis* B298 on controlling main diseases of red-chilli

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Formulation of microencapsulant *Bacillus subtilis* B298 antagonist biopesticide aimed for its high stability therefore have long viability more effective in controlling diseases, practical in transportation and application, economical and of course enviromentally friendly. This study was assessing the effectiveness of microencapsulant *B. subtilis* B298 antagonist biopesticide on controlling the main diseases of red chilli diseases (anthracnose and curly diseases) using microencapsulant formulation of maltodexstrin:gom arab ratio of 3:2. The study resulted the increased resitance of chilli plant to the diseases which showed in increasing of total phenol and flavonoid inhibition by 21.56% and 50.61% respectively. This can lead to decreased anthracnose disease in chilli peppers; which provide the less intensity of the disease in treatment using combination of microencapsulant *B. subtilis* B298 and fungicide compared to control.

Keywords: biopestiscide, Bacillus subtilis B298, *red-chilli, anthracnose and inhibition*

INTRODUCTION

Based on demand and availability, chilli is considered as a priority crop to be developed. Constraints in its cultivation is the presence of diseases which can reduce the yield quality and quantity. Pathogen, one of the biological agents, including food-borne, could threaten food safety; such as *Salmonella, Escherichia coli, Listeria dan Vibrio* (Sylvesteret *et al.* 2013). Food safety can be concentrated on healthy food sources, and the sustainable availability of foodstuffs from their cultivation until producing healthy food products, not contaminated by microbes.

Diseases in chilli are; anthracnose caused by fungus *Colletotrichum* sp., leaf spot by *Cercospora capsici*, and bacterial wilt by *Ralstonia solanacearum*, yellow by Gemini virus, curly by aphid as virus vector. The main and always present disease in chilli plant is anthracnose, curling, wilting, leaf spot and stem rot (Agrios, 2005; Saxena *et al.*, 2016).

Yield loss as resulting from *Begomovirus* infection ranges from 20-100% and its impact on economically losses suffered by farmers can reach billions of rupiah (Gaswanto *et al.*, 2016).

An alternative control of plant diseases that more effective, environmentally friendly and maintain food safety is the use of bacterial antagonists *Bacillus subtilis* B298. *B. subtilis* B298 is an antagonistic bacteria obtained from healthy potato rhizosphere; is a safe bacteria for plants and can even increase plant growth and resistance because it produces siderophores, IAA, as a phosphate solvent and produces antimicrobial compounds such as chitinase, protease, and amylase (Prihatiningsih *et al.*, 2015; Prihatiningsih and Djatmiko, 2016, Lestari *et al.*, 2017).

The microencapsulation formula is an innovation in biopesticide formulation with the objective of a more stable active ingredient of *B. subtilis*, longer viability and effectiveness. In the microencapsulation formula, *B. subtilis* will have higher viability than in liquid formula (Shekhar *et al.*, 2010).

Food safety can be viewed from the availability of healthy food, avoid the symptoms of diseases and maintained the quality of food, and safe consumption. Food safety is supported by a healthy plant in this case the management of pathogens, including foodborne pathogens, materials and processes as well as contamination (Hanning *et al.*, 2012).

Systemic resistance is systemically induced resistance due to changes in secondary metabolite content of plants such as total phenol compounds in plants in the form of flavonoids, alkaloids, tannins, terpenoids, saponins and glycosides which may poses an antioxidants and antimicrobial activities (Baba and Malik, 2015). The compounds produced from the extraction of plants containing secondary metabolites are thought to have antioxidant activity, and may further increase plant resistance to pathogens by the mechanism of induced systemic resistance.

MATERIALS AND METHODS

Material used are; *B. subtilis* B298, ingredients of the biopesticide formulation are maltodextrin and gom arab, pepper plant. Media used for *B. subtilis* B298 growth is YPGA (5g yeast extract, 10g pepton, 10g glucose, 20 g agar and 1000 ml of water).

Test for systemic resistance improvement

The plants ability to produce antioxidant compounds detected using DPPH method (1,1-diphenyl-2-picrylhydrazyl) (Tekao *et al.*,1994). The plant extract in methanol solution of 1 mg/mL. Dilution is made to obtain concentrations of 500; 250; 125; 62.5 and 31.25 μg/mL. Each was taken 1 mL to be dissolved into 1 mL of methanol solution of 1mM DPPH at concentration of 1 mg/mL; incubated for 30 minutes in the dark at room temperature then the absorbance measured at 517 nm.

The total phenol calculation conducted by calculating the inhibition percentage as an antioxidant activity with the formula of:

$$\% \text{ inhibition} = \frac{(\text{Absorbance blank} - \text{Absorbance of sample})}{\text{Absorbance blank}} \times 100\%.$$

The the inhibition percentage indicates the ability of the compound as an antioxidant which capable of inhibiting free radicals. The standard antioxidant of ascorbic acid as control is made with concentrations of 25, 50, 100, 200 and 400 ppm.

Test of major chilli disease control

Biopesticide applications based on *B. subtilis* B298 to control the main chilli disease were conducted with treatment on the seed by coating and followed by watering and spraying around the plants to control the disease on the ground.

The treatment are K: control without biopesticide; B: with biopesticide *B. subtilis* B298; F: with propineb-based fungicide; and BF: combination of biopesticides and fungicides. Biopestisida watering application of 100 mL per plant was done with interfal of 10 days starting at age of 20 hst (day after planting), while spraying was done with 7 days interfal.

The density of *B. subtilis* B298 used of 10^8 cfu/g formulation. The observed variable was the intensity of anthracnose disease with formulation according to Jeyalakshmi and Seetharaman (1998) that:

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

IP: disease intensity; n: the number of sick fruits per disease category; v score category of disease (from 0-5); Z: highest disease category (5); N: number of pepper fruit observed per plant. Score category of disease as follows 0: no symptoms of disease; 1: 1-5% area of chilli pepper covered by symptoms disease; 2:> 5-10% fruit area covered by symptoms of disease; 3:> 10-25% fruit area covered by symptoms of disease, 4:> 25-50% fruit area covered by symptoms of disease and category 5:> from 50% fruit area covered by symptoms of disease.

In leaf-infected pests with curly-like symptoms using the same formula but with the category of attack used as proposed by Santos *et al.* (2009): 0: healthy plants (asymptomatic); 1: <1% of symptom-leaf area; 3: 1-5% symptom-leaf area; 5: 6-25% symptom-leaf area; 7: 26-50% symptom-leaf area; 9:> 50% of the symptom-leaf area. Intensity of yellow attack using the same formula but with different categories ie: 0: no symptoms, 1: yellow leaves on the edge starting on young leaves, 2: all leaves almost yellow and slightly curly, 3 : leaves are yellow and curly and curved upward, leaves are smaller and plants are still growing, 4: dwarf plants and yellowing, small and growth has stalled. The IP values obtained are then used to group the genotype security level against *Begomovirus* by criteria of (Ganefianti *et al.*. 2008; Trisno *et al.*., 2010): Immune (I) if IP=0.00%, Resistance (T) if IP $\leq$ 10%, moderate resistance (AT) if 10% <IP $\leq$20%, moderate susceptible (AR) if 20% <IP$\leq$ 30%, susceptible (R) if 30% <IP $\leq$ 50%, high susceptible (SR) if IP> 50%.

RESULT AND DISCUSSION

The plant resistance to disease was increased by increasing total phenol and flavonoids compounds. The systemic resistance induction of plant to disease is one of application mechanism of biopesticide in indirect growth of plant growth. Increased total phenol and flavonoid compounds occurred in fruit respectively by 21.56% and 50.61% in *B subtilis* B298-Fungicide treatment (Table 1).

Table 1. Resilience of plant response after application of *B. subtilis* B298 and Fungicide

Treatment	Phenol inhibition (%)			Flavonoid inhibition (%)		
	Head	Roots	Fruit	Head	Roots	Fruit
Control	48,51	16,78	65,26	56,39	**53,59**	110,41
B subtilis B298	51,39	19,33	72,91	43,14	51,13	140,19
Fungicide	**52,97**	**23,02**	57,30	**73,27**	40,67	96,24
B subtilis B298-Fungicide	52,94	19,84	**83,20**	58,00	45,52	**223,53**
Increase (%)	8,41	27,19	**21,56**	23.03		**50,61**

Control of chilli major diseases after application of biopesticides and fungicides can be seen in Table 2, that shows application of Biopesticide *B.subtilis* B298 able to suppress anthracnose disease intensity with effectiveness equal to 53,94%, but to curly and yellow diseases only 14,14 % and 6.81%.

Table 2. The effectiveness of chilli major diseases control after application of *B. subtilis* B 298 dan Fungicide

Treatment	Yellow disease intensity(%)	Effectivity (%)	Anthracnose intensity (%)	Effectivity (%)	Curly disease intensity (%)	Effectivity (%)
Control	22,88	-	16,98	-	9,9	
B.subtilis B298	21,32	**6,81**	7,82	**53,94**	8,5	**14,14**
Fungicide	25,9	-	10,51	38,10	13,5	-
B.subtilis B298-Fungicide	36,5	-	13,78	18,84	9,5	4,04

This suggests that *B. subtilis* B298 as an active biopesticide ingredient has only control pathogenic fungi not control virus. This condition is supported by the ability of *B. subtilis* B298 to produce secondary metabolite in the form of chitinase enzyme capable of degrading fungal cell wall with activity equal to 6,145 U/mL, 6,937 U/mL and 5,764 U/mL; respectively at pH 5, incubation time 15 hour, and incubation temperature of 40°C (Lestari *et al.* 2017).

Phenol compounds including flavonoids in plants related to the level of plant resistance to pathogens. The higher the total phenol content related to its higher resistance. According to Iswanto *et al.* (2016) that plant resistance is affected by its secondary metabolite compounds produced, with different types and concentrations in each plant. For example, rice crops resistant to BPH (Brown Plant Hopper) are associated with its secondary metabolite compounds; such as oxalic acid, Tricin, Schaftoside, Isoscaftoside and Apigenin-C glycosides, which have an effect on BPH as deterrence, antifeeding and toxicosis. Gunaeni *et al.* (2015) stated that there are two types of plants namely **pagoda** plants (*Clerodendrum japonicum* Thunb.) and **tapak dara** (*Catharanthus roseus* L.) that can induce chilli plant resistance against yellow viral disease by showing chilli leaf thickness 1.5 times more than those not treated chilli plants and produced salicylic acid as a secondary metabolite. Chilli plant induced with **pagoda** and **tapak dara** plants extract higher by 53.99-134,38%. Plant extracts can exhibit antioxidant activity which can be function as an inducing plant resistance to pathogens (Praditasari, 2016).

The conclusions of this study are increased chilli plant resistance to pathogens indicated by the increase of total phenol and flavonoid by 21.56% and 50.61%; respectively. Biopesticide *B. subtilis* B298 application can suppress the intensity of anthracnose and curly disease. Biopesticide of microencapsulation formula gives the best result in suppressing anthracnose chilli disease by 53,94%.

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