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The Control of Panicle Blight Bacterial Pathogen on Rice Seeds Through *In Vitro* Treatments

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ABSTRACT

Burkholderia glumae is a seed-borne pathogen of rice known to cause bacterial panicle blight disease. The lack of effective control methods makes seed treatment the alternative management approach. The aim of this research was to determine an effective seed treatments technique, using liquid smoke, clove oil, hot water and copper hydroxide fungicide treatment against bacteria *B. glumae*. The experiment used a complete randomized design with five treatments and three replications, including control, liquid smoke, clove oil, hot water, and copper hydroxide fungicide. The results showed the propensity for all treatments to reduce bacterial populations on rice seeds, while liquid smoke, clove oil, and fungicide did not reduce vigour and viability. Application of copper hydroxide fungicide 77% at concentration of 5% was recommended as the best treatment to control the bacterial pathogen.

Keywords: *Burkholderia glumae*, clove oil, copper hydroxide fungicide, hot water, liquid smoke, rice

INTRODUCTION

Rice has been an important staple food crop for over half of the world's population, including Indonesia (Ishaq *et al*., 2017). However, extreme weather conditions in the form of floods, droughts and the outbreak of pests and diseases have been affecting the crop productivity (Lamichhane *et al.*, 2015). In Indonesia, *Burkholderia glumae* Urakami *et al*. (1994) is the important causal bacterial pathogen of rice panicle blight disease or bacterial grain rot disease, causing severe infection, and reducing the rice yield up to 75% (Trung *et al.* 1993). Recently, the occurrence of panicle blight disease has been reported in several locations, including West, Central and East Java (Wiyono *et al*., 2017), as well as South Sulawesi (Baharuddin *et al*., 2017) and North Sumatra (Hasibuan *et al*., 2018). According to the Minister of Agriculture, Republic of Indonesia, *B. glumae* is categorized as an A2 quarantine plant pest, which is impossible to remove from the seeds through quarantine treatments (Quarantine Agriculture, 2015). Since the potential for seed-borne pathogens dispersal via seed trade is high, the use of clean seed is recommended as an initial management approach to control the rice panicle blight disease (Suryani, 2017). For example, dipping in liquid smoke (a natural product made from condensation or pyrolysis of materials containing lignin, cellulose, hemicellulose and other carbon compounds), clove oil as well as hot water treatment, in order to reduce or eliminate the pathogens (Taylor & Dye, 1976; Zagory & Parmeter, 1984; Milus, 1997; Light & van Staden, 2004; Situmeang, 2013; Belmar *et al.*, 2014; Achrom, 2015; Spadoro *et al*., 2017; Aisyah *et al*., 2018).

This study was aimed at testing the potential of several seed-treatment methods, including liquid smoke, clove oil, and hot water, to control *B. glumae* on rice seeds.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Plant Disease, Faculty of Agriculture, Universitas Sumatera Utara, Indonesia from May to October 2018.

Bacterial isolate of *B. glumae* (IRC PRC) was obtained from the collection at Laboratory of Plant Disease, Faculty of Agriculture, Universitas

Sumatera Utara, which was confirmed by the previous study (Hasibuan *et al*., 2018), cultured on King's B medium, and incubated at 37 °C for 48 h. The bacterial suspension in sterile distilled water was measured using a spectrophotometer in order to attain 10^8 cfu/ml (OD 0.5, λ = 600 nm). Conversely, the rice seeds were surface sterilized using NaOCl for 5 minutes, washed with sterile distilled water, and air-dried on sterile filter papers, followed by introduction into the bacterial suspension (10^8 cfu/ml) and agitation for 4 h at room temperature. Then, the inoculated seeds were air-dried in a laminar air flow cabinet for 45 minutes and left overnight (a minimum of 15 hours) before the treatments.

All inoculated seeds (100 seeds per treatment) were treated with the following treatments for 10 minutes:

- Sterile distilled water as control,
- liquid smoke [5% and 7%, (v/v)],
- clove oil [2% and 5%, (v/v)],
- hot water (50 °C and 60 °C),
- Copper hydroxide fungicide 77% [2% and 5%, (v/v)].

The concentration and temperature for each treatment were selected on the basis of previous studies (Tung & Serrano, 2011; Situmeang, 2013; Achrom, 2015; Hoerussalam *et al.*, 2017)

The bacterial count was subsequently determined by dipping the seeds in phosphate-buffered saline (PBS) solution, and ground to attain 80% crushed samples. Then, the samples were suspended in 20 ml NaCl for 1 h at room temperature and agitated for 2 h. Next, the suspensions were subjected to a 10-fold dilution series, plated on King's B medium, and incubated at 37 °C for 48 h. The bacterial colonies formed were counted.

After the seeds were treated, the seeds were germinated and assessed for seed vigour and viability. The seed vigour was assessed by evaluating the percentage of healthy seedlings grown five days after planting according to Eq. (1).

$$\text{VI} = \frac{\Sigma \text{ NS 5 dap}}{\Sigma \text{ seeds planted}} \times 100\% \qquad (1)$$

Note:
VI : Vigour Index; NS: normal seedlings; dap: day after planting

Seed viability assessment involved the evaluation of total healthy seedlings percentage 5 day- and 14 day-after planting according to Eq. (2).

$$\text{VI} = \frac{\Sigma \text{ NS 5 dap} + \Sigma \text{ NS 14 dap}}{\Sigma \text{ seeds planted}} \times 100\% \qquad (2)$$

Note:
VI : Vigour Index; NS: normal seedlings; dap: day after planting

(Sutopo, 2004)

The data obtained were evaluated using Analysis of Variance for non-factorial Completed Block Design with three replications and Duncan's Multiple Range Test with SPSS Software to ascertain the possibility of treatments significantly affecting other parameters.

RESULTS AND DISCUSSION

The bacterial count were reduced after the treatments compared to the initial values recorded (10^8 cfu/ml), after a 48 h incubation (Table 1). All treatments were able to reduce bacterial population by up to 99%, as compared to the initial bacterial suspension concentration. Furthermore, all treatments significantly reduced the *B. glumae* bacterial count when compared to the control treatment. The higher concentration in all treatments resulted lesser bacterial count as compared to the lower concentrations. Therefore, the higher concentrations of each treatment was associated with the visibly reduced bacterial colonies, where fungicide at the concentration of 5%, together with the two concentrations of liquid smoke demonstrated the most significant effect.

The best seed vigour (95.57% and 94.53%) was observed with fungicide, at the respective concentration of 2% and 5%, which was not different from the control and as well as from the liquid smoke treatments (Table 2). Table 2 shows the outcome for clove oil and hot water treatments, which was not good, as 2% and 5% clove oil resulted in <80% of seeds capable of normal growth, based on the Indonesian standard quality requirements, which is more than 90% (Directorate General of Food Crop, 1991). Furthermore, none of the seeds were germinated in hot water treatment (Table 2).

Although there was no significant difference with the conrol treatment, rice seed germination rate was as high as control treatment (>80%) for the

liquid smoke and fungicide treatments. Conversely, the germination rate was at 75.43% and 56.66%, when treated with clove oil at the respective concentration of 5% and 2%. The use of selected concentrations of clove oil were on the basis of previous studies (Kishore & Pande, 2007; van der Wolf *et al*., 2008; Hoerussalam., 2017), which applied not more than 5% of clove oil concentration as seed treatment for various crops. However, the rice seeds became toxic and could not germinate when the seeds were treated by clove oil at concentration of 2%, 3% and 4%. Also, 50 °C and 60 °C hot treatments were unsuitable alternatives, because almost all seeds were not germinated (Table 2). The rice seed germination with treatments as seen in Figure 1.

Copper hydroxide fungicide and liquid smoke were able to reduce the population of *B. glumae in vitro*. This was possibly due to the presence of active ingredients in the form of phenol compounds, organic acids, and also copper, which particularly inhibits bacterial growth by binding to and hindering the synthesis of proteins in bacterial cells (Aisyah *et al*., 2018). Therefore, errors are generated while reading the genetic codes, ultimately leading to bacterial cell death (Hikichi & Egami, 1998). The liquid smoke treatment is applied to suppress several plant diseases, based on the effective antimicrobial (Lingbeck *et al*., 2014) antioxidant and antibacterial activities (Yang *et al*., 2016). The major antibacterial compounds detected in liquid smoke include 2,6-dimethoxyphenol (syringol, 29.54%), 2-methoxyphenol (guaiacol, 12.36%), and 3,5-dimethoxy-4-hydroxytoluene (11.07%) (Yang *et al*., 2016). In addition, there are also a great potential for application as an organic pesticide and herbicide (Payamara, 2011).

Liquid smoke confers a positive effect in seed germination and seedlings vigor on a wide range of plant species (Abdollahi, 2012; Flematti *et al*., 2011). These activities are possibly due to the chemical composition, including catechol, which has previously been used for promoting *Nicotiana attenuata* root growth (Wang *et al*., 2017).

This current study showed a bad performance for hot water as a seed treatment to promote germination, as the heat generated does not break dormancy, but causes damages, especially in the embryo, which inhibits seeds growth. The hot water treatment at 54 °C for 10 minutes caused retardation and reduction in wheat seed germination due to seed coat breakage (Tapke, 1923). Meanwhile, the introduction of hot water at 55 °C for 25 minutes on tomato seeds infected by *Clavibacter michiganensis. michiganensis* (Smith) lead to a low germination rate of 52.22% (Nalis, 2015). This also occured after introducing corn seeds infected by *Pantoea stewartii subsp. Stewartii* to temperatures above 50 °C (Nalis, 2015).

Table 1. Bacterial population on rice seeds infected by *B. glumae* after different treatments

Treatments	Bacterial population (cfu/ml)
A0 (Control)	5.04×10^{8} [a]
A1 (Liquid smoke 5%)	3.26×10^{3} [e]
A2 (Liquid smoke 7%)	3.16×10^{3} [e]
A3 (Clove oil 2%)	4.69×10^{3} [b]
A4 (Clove oil 5%)	4.32×10^{3} [c]
A5 (Hot water 50°C)	4.70×10^{3} [b]
A6 (Hot water 60°C)	4.32×10^{3} [c]
A7 (Fungicide 2%)	3.89×10^{3} [d]
A8 (Fungicide 5%)	3.10×10^{3} [e]

Table 2. Post-treatment vigour and viability of rice seeds infected with *B. glumae*

Treatments	Seed vigour (%)	Seed viability (%)
A0 (Control)	89.35[a]	93.40 [ab]
A1 (Liquid smoke 5%)	90.86 [a]	95.88 [ab]
A2 (Liquid smoke 7%)	87.63 [a]	88.21 [b]
A3 (clove oil 2%)	30.52 [c]	56.66 [d]
A4 (Clove oil 5%)	58.33 [b]	75.43 [c]
A5 (Hot water 50 °C)	0.00 [d]	0.00 [e]
A6 (Hot water 60 °C)	0.56 [d]	0.57 [e]
A7 (Fungicide 2%)	95.57 [a]	96.96 [ab]
A8 (Fungicide 5%)	94.53 [a]	99.14 [a]

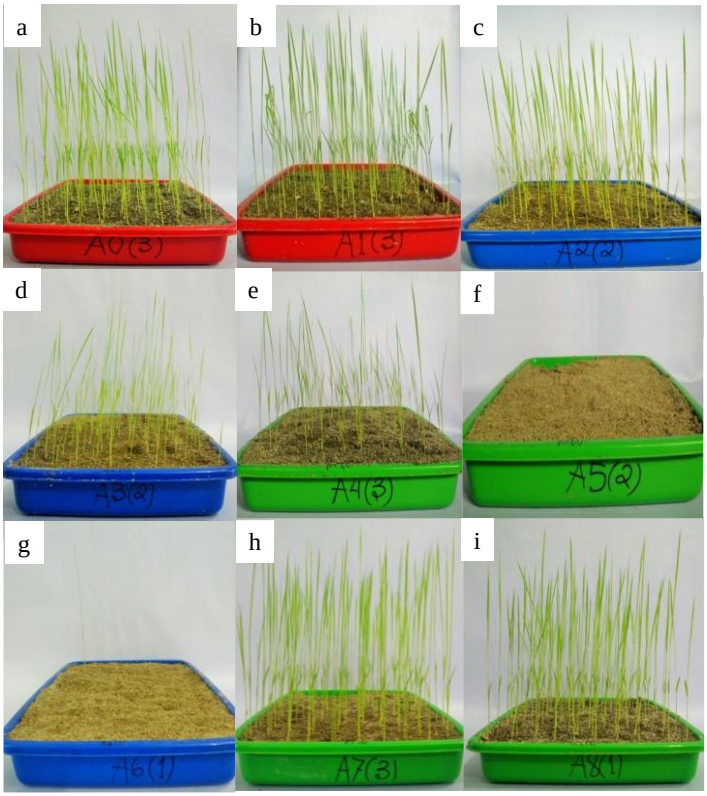

Figure 1. The germination of infected rice seeds with *B. glumae* after treated in the following treatments a) control; b) 5% liquid smoke; c) 7% liquid smoke; d) 2% clove oil; e) 5% clove oil; f) hot water at 50 ^{o}C; g) hot water at 60 ^{o}C; h) 2% fungicide; i) 5% fungicide

CONCLUSION

The application 5% copper hydroxide fungicide 77% could control *B. glumae* population on rice seeds. This treatment was confirmed to confer a positive influence on the vigour and viability of seeds.

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