



The Effect of Volume and Inoculation Method on the Development of Bacterial Panicle Blight Disease in Rice Plant

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ABSTRACT

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Bacterial panicle blight in rice plants caused by *Burkholderia glumae*. These bacteria interfere with the grain filling process so that the grain is not filled and can cause yield losses of up to 75% on pathogenic infested land. This study was conducted to determine the effect of *B. glumae* suspension volume and inoculation method on the development of bacterial panicle blight. The research was conducted on vegetative and generative rice plants using a factorial completely randomized design with the volume of bacterial suspension as the first factor and the inoculation method as the second factor. The results showed that the higher the volume of the *B. glumae* suspension inoculated, the higher the severity of bacterial panicle blight in rice plants. Both the inoculation and injection methods can cause the same disease severity. Besides, the severity of bacterial panicle blight in the generative phase is more severe than in the vegetative phase.

INTRODUCTION

Until now, rice is still the main source of nutrition and energy for more than 90% of Indonesia's population. In 2014, Indonesia's total rice production was 70.84 million tons and in 2015 it was 75.39 million tons (Central Bureau of Statistics 2018b) from 8.1 million hectares (Central Bureau of Statistics 2018a). The average level of rice consumption in Indonesia is 111.58 kg/capita/year, the demand for rice in 2020 and 2025 is estimated to reach 62.3 million tons and 65.8 million tons. The population that continues to grow and there is no indication of a decrease in rice consumption is one of the challenges. Besides that, the

presence of pests and pathogens is one of the limiting factors in rice cultivation. One of them is panicle blight caused by bacteria caused by the *Burkholderia glumae* bacteria.

B. glumae is a gram-negative bacteria that was an important pathogen in rice in Japan which can cause grain rot, stem rot, and seed rot, also known as panicle blight (Nandakumar *et al.* 2009). The presence of this disease then began to be reported causing severe damage in various countries, such as in Taiwan, the United States, China, and South Africa. *B. glumae*, which was previously only a minor pathogen, has increased its status to become the main pathogen in rice, this is related to changes in conducive weather, namely warm

nights and high humidity and rainfall during the growing season. Besides, the use of hybrid varieties that have high yield potential but tend to be more susceptible to disease is also one of the causes for the emergence of new diseases (Joko 2017).

In 1987, the presence of this disease had been reported in Indonesia as stated in the press release of the Ministry of Agriculture, but after that, it was not yet reported any further damage due to this disease until then the existence of this disease began to be reported back since 2015 in several areas in Indonesia (Joko 2017), especially in some rice centers in the Java region (Handiyanti *et al.* 2018).

B. glumae is a seed-borne pathogen and can cause yield losses of up to 75% in pathogen infested land (Zhou-qi *et al.* 2016). The decrease was caused by inhibition of germination, seed blight, stem rot, sterile florets, and abnormal grain (Karki 2013). Symptoms of *B. glumae* infection appear in the midrib form of long greyish patches with reddish-brown edges. Another characteristic is panicle growing up and yellowish with the base of the flower is dark and there is a reddish-brown line that crosses in the middle. At high infection rates, the filling process can be interrupted. This causes the panicles to grow upright because the grains are not filled. Panicle blight disease has typical symptoms, which often occur sporadically in a plant or a circular or oval pattern on a planted area. This is different from empty panicle diseases which are usually caused by abiotic stress such as excessive heat, symptoms will appear more uniform and not circularly patterned (Nandakumar *et al.* 2009).

B. glumae bacteria produce phytotoxins, namely toxoflavins. Toxoflavins which play a role in pathogenicity in infecting rice plants. Toxoflavin can cause inhibition of the growth of shoots and roots in rice seeds and also causes grain rot in rice panicles (Joko 2017). This research was carried out to determine the effect of *B. glumae* suspension volume and inoculation method on the bacterial panicle blight disease severity.

MATERIALS AND METHOD

Time and Place

The research was conducted from March to May 2019 at the Laboratory of the Plant Protection Department, IPB University.

Isolation of *B. glumae* bacteria

B. glumae isolate is a isolate from the collection of Widarti *et al.* (2020). Isolation of *B. glumae* culture was carried out on nutrient agar (NA) media and King's B media. King's B media was carried out to see the activity of toxoflavin released by *B. glumae*.

Propagation of *B. glumae* Bacterial Suspension

Propagation of *B. glumae* bacterial suspension was done by taking 1 ose of bacterial inoculum and cultured in luria broth (LB) media, then the media was shaken using a shaker with a speed of 150 rpm for 48 hours.

Testing of *B. glumae* Bacteria in Vegetative Phase of Rice Plants

The inoculation of *B. glumae* bacteria in the 21-day-old vegetative rice plant was carried out by the method of injection on the plant midrib. The experimental design used was a completely randomized design (CRD) with each treatment consisting of 4 replications. The details of the treatment are as follows:

1. Injecting *B. glumae* volume of 0 ml/plant
2. Injecting *B. glumae* volume of 0.5 ml/plant
3. Injecting *B. glumae* volume of 1 ml/plant
4. Injecting *B. glumae* volume of 1.5 ml/plant

After inoculation, the scar was covered with plastic for 3 days to maintain the humidity of the plants.. Observations were made up to 6 weeks after inoculation (wai) by measuring the size of the lesion.

Testing of *B. glumae* Bacteria in Generative Phase of Rice Plants

Inoculation of *B. glumae* bacteria in the 64-day-old generative rice plant was carried out by two different methods, namely the injection method on the stem and spraying on panicle candidates. The experimental design used was a completely factorial randomized design with the first factor being the volume of bacterial

suspension and the second factor was the inoculation method. Each treatment consisted of 4 replications. The details of the treatment are as follows:

1. Injecting *B. glumae* volume of 0 ml/plant
2. Injecting *B. glumae* volume of 2 ml/plant
3. Injecting *B. glumae* volume of 3 ml/plant
4. Injecting *B. glumae* volume of 4 ml/plant
5. Spraying *B. glumae* volume of 0 ml/plant
6. Spraying *B. glumae* volume of 2 ml/plant
7. Spraying *B. glumae* volume of 3 ml/plant
8. Spraying *B. glumae* volume of 4 ml/plant

After inoculation, the rice plants were covered in plastic for 3 days to maintain the humidity of the plants. Observations were made up to 6 wai with the following disease

Table 1. Scoring severity of *B. glumae* bacterial panicle blight disease (Karki *et al.* 2012)

Score	Damage Scale
0	There are no symptomatic plants
1	10% symptomatic plants
2	11%-20% symptomatic plants
3	21%-30% symptomatic plants
4	31%-40% symptomatic plants
5	41%-50% symptomatic plants
6	51%-60% symptomatic plants
7	61%-70% symptomatic plants
8	71%-80% symptomatic plants
9	> 80% symptomatic plants

scale criteria (Table 1).

Based on the results of the damage scale assessment, the disease severity was calculated using the Townsend and Heuberger formula (1974 in Sinaga 2006) as follows:

$$\text{Disease severity} = \frac{\sum_{i=1}^n (n_i \cdot v_i)}{N \cdot V} \cdot 100\%$$

Where n, v, N, and V were number of plants

attacked in category I, category damage number-I, number of plants observed, and highest number of attack categories, respectively.

RESULTS AND DISCUSSION

Morphological Characteristics of *B. glumae* Bacteria

Morphological observations of *B. glumae* isolates showed that the colonies had smooth, convex, cream-white edges on NA media. When virulent colonies grew on King's B medium, they formed a yellow pigment but did not fluoresce under ultraviolet light (Figure 1). According to Schaad *et al.* (2001), the *B. glumae* colony has its uniqueness when planted in the media so that it is non-fluorescent and diffuses. Colony color with diffuse greenish yellow pigment similar to *Pseudomonas fluorescent* colony. However, the *P. fluorescent* colonies that grew on King's B media would produce luminous colors when observed under ultraviolet light, while the *B. glumae* colonies did not produce fluorescence.

Pathogenic Burkholderia isolates generally produce yellow pigments identified as toxoflavins, while non-pathogenic isolates do not produce yellow pigments (Nandakumar *et al.* 2009). Yellow pigment plays a role in the pathogenicity of *B. glumae* in infecting host plants. *B. glumae* bacteria produce phytotoxic toxoflavins which play an important role in its virulence. The resulting toxoflavin was 1,6-dimethylpyrimidio [5,4-e] -1,2,4-triazine-5,7 (1H, 6H) -dione. This compound has a molecular weight of 193 (Jeong *et al.* 2003). Strains that cannot produce toxoflavin to avirulent (Nandakumar *et al.* 2009). The role of toxofla-

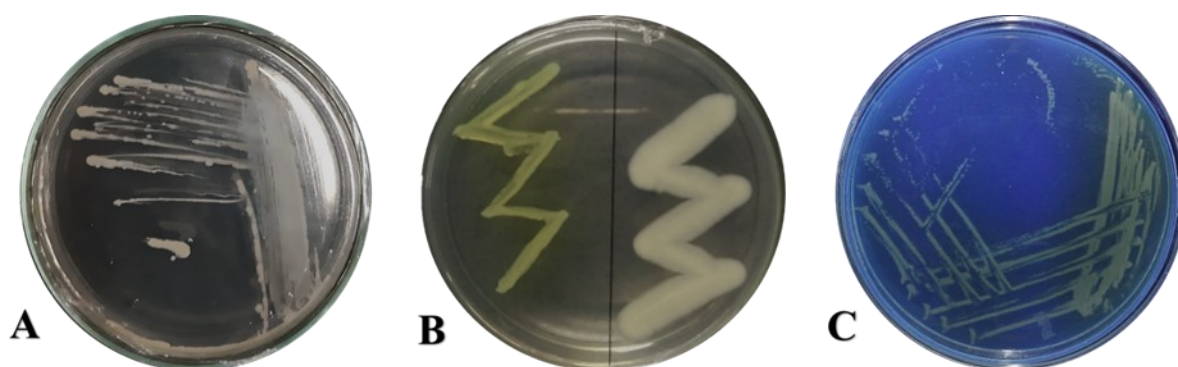


Figure 1. (A) *B. glumae* colonies on NA media, (B) *B. glumae* colonies (left) and *Bacillus cereus* (right) on King's B media, and (C) *B. glumae* colonies on King's B media under UV

vine in pathogenicity is indicated by the symptoms and the severity of the resulting disease. Toxoflavin can inhibit several cellular and metabolic processes in plants (Jeong *et al.* 2003).

Testing of *B. glumae* on Vegetative Phase of Rice Plants

The results showed that the higher the volume of *B. glumae* suspension injected into the midrib of rice plants, the greater the size of the lesion at the injection site. However, the size of the lesion in each treatment volume of *B. glumae* suspension was not significantly different between treatments, compared to the control treatment without inoculation of the *B. glumae* suspension. The lesion size in the injection-treated plants did not change since the 2 treatments (Table 2).

Inoculation of bacterial suspensions in one-month-old rice plants with a minimum of 4 leaves causes linearly shaped lesions on the stem sheath with clear reddish-brown and the grey border with the central part of the necrosis, causing rot of the sheath and stem rot.

On the leaves, the lesions are circular to oval in shape with the tip of the leaf containing white blight, over time the leaves will dry out and fall out. The same thing was stated by Cottyn *et al.* (1996), infected rice seedlings will grow smaller and have brown rot on the midrib, and the leaves undergo morphological changes to be curly, curved, and white at 1 to 2 wai. (Figure 2).

Testing of *B. glumae* on Generative Phase of Rice Plants

The results showed that the higher the volume of *B. glumae* inoculated in rice plants, the higher the disease severity. Disease severity values between treatment volumes are significantly different. The disease severity ranging from 41% to 69% at 6 wai (Table 3).

Based on studies was conducted by Li *et al.* (2016) with the gfp gene marker, initially, *B. glumae* was in rice plants before panicle formation. Pathogenic bacteria initially surround the glume surface and colonize until the population increases in the glume hairs.

Table 2. Development of lesion size in vegetative rice plants

No	Treatment	Size of Lesion (cm) at week-*			
		1 wai	2 wai	4 wai	6 wai
1	<i>B. glumae</i> volume 0 ml	0.00 a	0.00 a	0.00 a	0.00 a
2	<i>B. glumae</i> volume 0.5 ml	0.58 b	0.63 b	0.63 b	0.63 b
3	<i>B. glumae</i> volume 1 ml	0.55 b	0.85 b	0.85 b	0.85 b
4	<i>B. glumae</i> volume 1.5 ml	0.58 b	1.00 b	1.00 b	1.00 b

* numbers in the same column followed by the same letter are not significantly different at the 5% test level (Duncan's multiple range test)

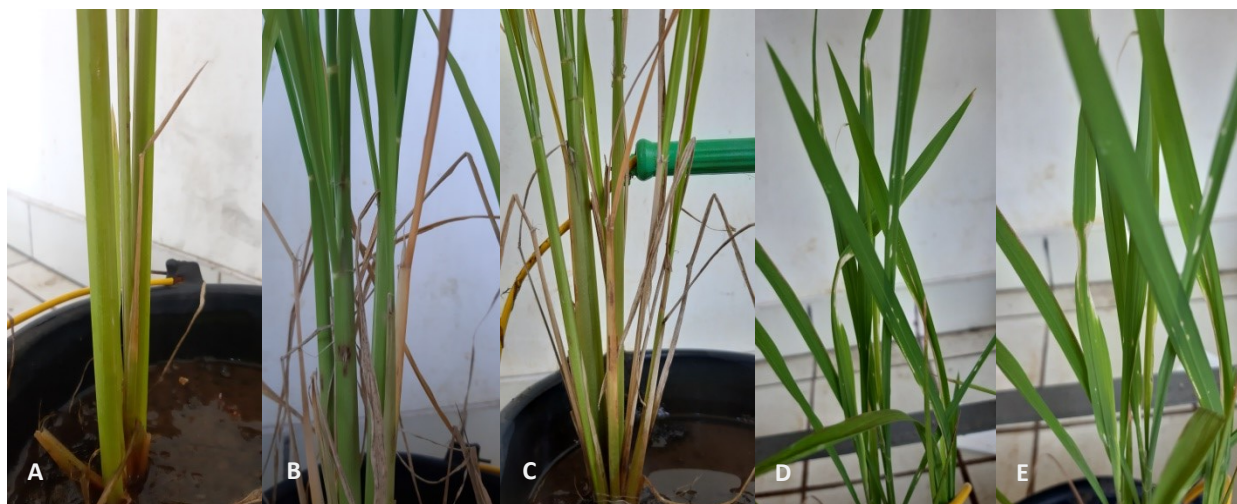


Figure 2. (A) rice stems in control treatment, (B and C) rice stems in a treatment injected with *B. glumae*, (D and E) rice leaves with symptoms of white blight

Table 3. Percentage of disease severity from several treatments of *B. glumae* suspension volume

Treatment	Disease Severity at week-*				
	1 wai	2 wai	3 wai	4 wai	6 wai
<i>B. glumae</i> volume 0 ml	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
<i>B. glumae</i> volume 2 ml	7.63 ab	20.88 b	34.38 b	38.13 b	41.25 b
<i>B. glumae</i> volume 3 ml	16.00 bc	28.75 b	42.50 b	48.13 c	53.75 c
<i>B. glumae</i> volume 4 ml	21.50 c	39.38 c	58.75 a	65.63 d	69.38 d

* numbers in the same column followed by the same letter are not significantly different at the 5% test level (Duncan's multiple range test)

Furthermore, penetration occurs to the inner surface of the palea and lemma, and spreads to the gynoeceum and stamen. Pathogenic bacteria carry out colonization in lodicules and ovaries, stylus, stigma, filament, and stamen. The bacteria then gather in cracks in the glume. Furthermore, bacteria begin to infect the pollen grains that cause deformation and abortion in the pollen grains. At the beginning of colonization, bacterial concentrations were only around 10^3 cfu/g which then increased dramatically to 10^8 cfu/g on the 10th day after inoculation.

The most vulnerable period for floret infection is during panicles and flowering. Inoculation during flowering gives the highest rate of floret infection and production of diseased spikelets (Shahjahan *et al.* 1998). Research conducted with emerged panicle inoculation showed that infection increased proportionally when an increase in the lognormal volume of inoculum density. Inoculum density of 10^2 cfu to 10^4 cfu with a volume of 1 ml is the minimum inoculum density when spraying in the field (Tsushima *et al.* 1985). Pathogen cells found in leaf sheath

play an important role in primary infection. The infection of the leaf sheath is the main source of inoculum to the panicles that appear. Bacterial movement is carried out through the vascular system (Tsushima *et al.* 1991 and Tsushima *et al.* 1996).

When viewed from the two methods of *B. glumae* inoculation in the generative phase of rice plants showed that there were no significant differences between the two inoculation methods. Both methods can cause disease severity values ranging from 11% to 40% in rice plants from 1 wai to 6 wai (Table 4).

The initial symptoms that appear on the rice grains are brown spots which then spread to all parts of the grains so that the grains become dry and hollow. The dry, hollow ears fall out, while the green ears begin to show brownish and hollow spots, then a few moments later fall out (Figure 3). The chlorotic symptoms of the panicle and grain rot and reduced leaf and root growth in rice seedlings are caused by the production of toxoflavin and ferverulin which play a very important role in the pathogenicity of *B. glumae* in rice plants (Jeong *et al.* 2003).

Figure 3. Symptoms development in grains of panicle after inoculation of *B. glumae*

Table 4. Percentage of disease severity from two methods of inoculation of *B. glumae* suspension

Treatment	Disease Severity at week-*				
	1 wai	2 wai	3 wai	4 wai	6 wai
Spraying method	11.56 a	23.88 a	32.50 a	37.81 a	41.56 a
Injection method	11.00 a	20.63 a	35.31 a	38.13 a	40.63 a

* numbers in the same column followed by the same letter are not significantly different at the 5% test level (Duncan's multiple range test)

Bacterial panicle blight has three characteristics that distinguish it from other panicle diseases, namely (1) this disease often does not appear to interfere with the pollination process, so that seeds can be found in panicles, in contrast to sterile panicles due to heat stress, (2) infected panicles experience the color changes from light green to brown at the base of the panicle, with a reddish brown color separating this part from the rest which will then be colored like straw, and (3) the panicles (rachis) remain green for a while at the base of the flower shop, even after the seeds -The dried grains will turn brown. Floret (seeds) is the last stage of infection which is generally seen gray or black due to the growth of saprophytic fungi, and symptoms of rot with a black line on the grain (endosperm) (Nandakumar *et al.* 2009).

Symptoms also appear on the fronds, namely longitudinal gray spots surrounded by brown spots (Figure 4). The severity of the disease in some of these treatments is supported by environmental factors, namely temperature.

The optimal growing temperature for *B. glumae* is around 30 ° C, it can even grow to 41 ° C. Toxoflavin production is temperature dependent and the growth reaches a maximum level at 37 ° C, but no toxoflavins have been detected to be produced at temperatures between 25°C and 28 °C (Zhou-qi *et al.* 2016).

CONCLUSION

From the results of the study, it can be concluded that the higher the volume of *B. glumae* suspension inoculated in rice plants, the higher the severity of bacterial panicle blight. Both the inoculation and injection methods can cause the same disease severity. In addition, the severity of bacterial panicle blight in the generative phase of rice plants was higher than the vegetative phase.

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Figure 4. Symptoms on the midrib (A) control treatment and (B) inoculation of *B. glumae* suspension

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