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The Existing Method to Assess the Disease Incidence of Bacteria Leaf Blight and Its Effect to Rice Yield to Support the Indonesian Agriculture Insurance

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Abstract

Bacterial leaf blight (BLB) has become a major obstacle and a limiting factor in national rice production. Basically, this disease is caused by the bacterium Xanthomonas oryzae pv. oryzae. Therefore, to minimize the risk of disease infection to yield lost; The Ministry of Agriculture of the Republic of Indonesia has the agriculture insurance program. To support the program, the observations of disease incidence of the disease using existing method are needed. The purpose of this study was to determine the development of bacterial leaf blight on rice plants in Subak Munggu and to determine the effect of bacterial leaf blight on the yield of rice plants. Research activities included (1) isolation of pathogens from symptomatic leaves of rice plants, (2) weekly observations (3) morphological identification of pathogens (4) yields analysis. The results of this study showed the development of bacterial leaf blight in Subak Munggu, Munggu Village, Mengwi Sub-district, Badung Regency for ten weeks. The yield by calculating the weight of grains in each plot was calculated, it can be concluded that a sample that showed a high percentage of disease at the harvest time will cause the low grain yield production.

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INTRODUCTION

Indonesian people make rice as the staple food, thus rice is a very important commodity in Indonesia (Abdullah, 2002; Husodo, 2004). In rice cultivation, one of the problems faced was pests and plant diseases problem, one of them was bacterial leaf blight (BLB). BLB in Indonesia was known to farmers as the "kresek" disease, the disease was caused by the bacterium *Xanthomonas oryzae* pv. oryzae (Xoo).

This disease was one of the main diseases of rice plants in rice producing countries in the world. The spread of the disease included in Japan, Taiwan, China, Korea, Vietnam, Thailand, Philippines, Sri Lanka, India, Africa, Australia, South America and Indonesia (Ou, 1985; Hifni & Kardin, 1998; Suparyono, Sudir, & Suprihanto, 2004; Semangun, 2004). BLB disease was first reported by Reitsman and Schure in 1950. The pathogen that caused bacterial leaf blight in Indonesia was the same as which attacked rice plants in Japan, so the name was changed to *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. In 1976, the name of this pathogen became *Xanthomonas campestris* pv. oryzae and since 1992 *named Xanthomonas oryzae* pv. Oryzae (Goto M, 1964). The initial symptom of BLB attack was the rice leaves turned pale yellow, then wilt, dry and die (Wahyudi, Meliah, & Nawangsih, 2011). The infection from these bacteria caused damage to the leaves, which had an impact on reducing the photosynthetic ability of rice plants and disrupting the grain filling process, so that the grain became empty (Mew, Cruz, & Rayes, 1982). This disease had a very large spread during the rainy season and very humid temperatures, so it needed attention in controlling efforts terms.

So far, one controlling methods that considered the most effective was by planting varieties of BLB resistant rice. However, the problem with this controlling method was the ability of the pathogen to form new pathotypes that have higher virulence, so that the resistance of these varieties was easily broken (Qi & Mew, 1989; Suparyono et al., 2004). Therefore, the use of resistant varieties in controlling BLB disease was being continuously developed to obtain an effective and efficient method (Ogawa, 1993).

The yield loss due to BLB disease has been quite high, especially in the rainy season, reached 20.6 - 35.6%, while it was lower in the dry season which reached 7.5 - 23.8% (BBPOPT, 2007). BLB disease that appeared in early growth resulted high yield loss, even resulted crop failure or zero crop (Reddy, 1989). Meanwhile, if the bacterial attack occurred during the flowering phase, the grain filling process was disrupted, caused the grain to be incompletely filled or empty. This condition resulted a yield loss up to 50-70% (Mew et al., 1982; Suparyono & Sudir, 1992; Elings et al., 1997). Based on this data, to reduce the risk of rice farmers' losses due to the BLB disease, the government created an agricultural insurance program

(Ministry of Agriculture of The Republic of Indonesia, 2019). In determining the damage caused by BLB attacks, pest observers from the relevant Agriculture Department performed direct methods or field observations. So far, the damage assessment has been conducted on irregular plots or only based on farmer reports, so that the disease development cannot be predicted and data of the effect of damage on crop yields were uncertain. Therefore, this study was conducted in regular plots with the following aims: 1) To determine the development of bacterial leaf blight (BLB) in rice plants in Subak Munggu, Mengwi; 2) To determine the effect of bacterial leaf blight on rice yields in Subak Munggu. The results of this study were very useful for developing an assessment of damage due to BLB, not only using direct field observations but using modern approaches such as drone and remote sensing to support agricultural insurance.

RESEARCH METHOD

Research Time and Location

The research consisted of field research and laboratory research. Field research to monitor disease development and its effect on crop yields was conducted in Subak Munggu, Mengwi Sub-district, Badung Regency, Bali Province. This research was conducted for three months from February 2019 to April 2019 (one planting season). The laboratory research to confirm the cause of BLB disease that found in Subak Munggu was conducted at the Plant Disease Laboratory, Faculty of Agriculture, Udayana University. The study was conducted by observing 30 samples of rice plants used a regular plot system in 3 observation plots. In each plot, 10 clumps of rice were taken diagonally and marked with a stake to observe the development of BLB disease once a week. The observation of regular terraces aimed to observe the development of BLB disease in rice plants (Ministry of Agriculture of The Republic of Indonesia, 2018).

Tools and Materials

The tools used in this study were as follows: microscope, deck glass, cover glass, tweezers, petri dish, scissors, sprayer, Erlemeyer glass, measuring cup, micro pipette, autoclave, laminary flow (sterilization), cutter, camera, GPS, chlorophyll meter, tissue, plastic bag, 1.5 m stake, stationery, and observation table. The materials used in this study were media of Nutrient Agar/NA, 70% and 90% alcohol, distilled water/sterilized water, rice plants with BLB disease symptoms.

Isolation of Pathogen that Caused Bacterial Leaf Blight (BLB)

The isolation of pathogens that caused BLB disease aimed to ensure that the cause of BLB disease was bacteria. Plant samples were taken from rice plants that showed illness symptoms such as: the leaves turned pale yellow, wilted, and then died. Isolation was performed by cutting the infected plant leaves with a size of about 1x1 cm, then they were immersed in 70% alcohol solvent for 2 minutes to remove contamination on the outside of the plant. The plant parts were then immersed in distilled water/sterilized water at least 3 times; then they were planted on the surface of NA media, incubated at room temperature for 3-5 days, and ensured that the cause of the disease was bacteria.

The Observation of Disease Symptoms and the Development of Bacterial Leaf Blight Disease

The observation of symptoms of bacterial leaf blight disease on rice plants was conducted directly in the field, by observing symptoms on the leaves. Observations of disease development were observed in each observations sample. The plant samples that observed included clumps and tillers of rice plants according to the level of attack, then noted in the observation table.

The percentage calculation of bacterial leaf blight disease used the non-absolute calculation of damage. Observations were made when rice had been attacked by BLB and then observed at intervals of a week until harvest (Ministry of Agriculture of The Republic of Indonesia, 2018), by using the formula according to (Herdiana, 2010) with slight modifications.

$$\text{PS } (\%) = \frac{Nh}{Nt} x\ 100$$

Description:
PS = Disease Percentage (%)
Nh = Total leaves that attacked in one observed clump
Nt = Total leaves in one observed clump

The Relation of Disease Percentage and Yields

The relation between the attacks percentage and yields used a regression formula, to find out how the independent variables/criteria can be predicted through independent variables or predictor variables, individually. The impact of using regression analysis can be used to decide whether the increase and decrease in the dependent variable can be done by increasing and decreasing the condition of the independent variable, or increasing the condition of the dependent variable by increasing the independent variable and vice versa. Y = subject in the predicted dependent variable, a = Y value if X = 0 (constant value), b = direction number or regression coefficient, which showed the increase or decrease in the dependent variable based on the independent variable. If b (+) then increase, and if (-) then there was a decrease, x = subject to the independent variable which has a certain value. Technically, the value of b was the tangent of (comparison) between the line length of the independent variable and the dependent variable, after the regression equation, the regression analysis formula and correlation were found (Sudjana, 1996). The calculation of leaf chlorophyll content was calculated using SPAD Konica Minolta according to the applicable protocol. These measurements were conducted to determine the effect of disease attacks with chlorophyll content.

$$\dot{\mathbf{Y}} = \boldsymbol{a} + \boldsymbol{b}\,\mathbf{X}$$

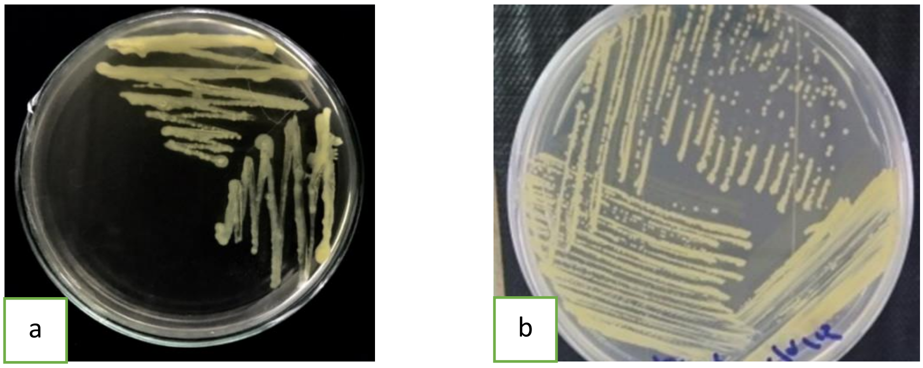
Description:

Y = Criterion Variable	a = Constant Variable
X = Predictor Variable	b = Coefficient of Linear Regression Direction

RESULT AND DISCUSSION

The Confirmation of Pathogen that Caused Leaf Blight Disease

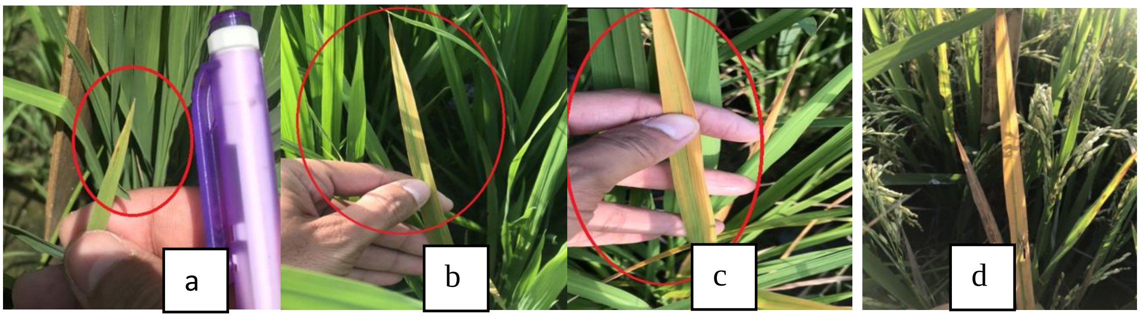
The pathogens' isolation from plants that showed blight symptoms aimed to ensure that leaf blight disease was caused by bacteria. The results of the isolation that had been conducted by breeding the symptomatic parts of the rice plant showed that the cause of the leaf blight disease was bacteria (Picture 1). Pure breed of isolated bacteria showed the following characteristics: bacterial colonies were round, convex, colored whitish yellow to straw yellow, with smooth surfaces and edges of colonies and sometimes dark, sometimes light. Colony diameter on agar medium was 1-2 mm as reported by Goto, 1990.



Picture 1. Bacteria colony resulted from the isolation of rice plants with leaf blight bacteria symptoms on NA media. (a) Bacteria breed on NA media resulted from isolation of infected plant. (b) Bacteria morphology of *Xanthomonas oryzae pv. oryzae* according to Jonit, Low, & G.H.Tan, 2016

The Symptoms of Bacterial Leaf Blight Disease

The symptoms of rice bacterial leaf blight in Subak Munggu showed typical characteristics of rice plants aged 28 days after planting (Picture 2a), 51 days after planting (Picture 2b), 71 days after planting (Picture 2c), and 88 days after planting (Picture 2d). Leaf margins of rice plants that infected with BLB turned brownish pale yellow. The changes in leaf color started from the edge of the leaf spread to the base of the leaf, while the center or leaf bone remained green. Symptoms of a severe attack began with rice leaves turned yellow, wilting, drying and then dying (Wahyudi et al., 2011). The bacterial infection resulted in the grain not being fully filled and empty. This was due to the disturbed photosynthetic ability of the rice leaves (Mew et al., 1982).



Picture 2. The symptoms of bacterial leaf blight (BLB) on rice plant. (a) Early symptoms of bacterial leaf blight on rice aged 28 days after planting (hst). (b)

Advance symptoms of bacterial leaf blight on rice aged 51 days after planting. (c) Disease symptoms spread to the leaf base on rice aged 71 days after planting. (d) Severe symptoms of leaf blight and already reach leaf base part on rice aged 88 days after planting.

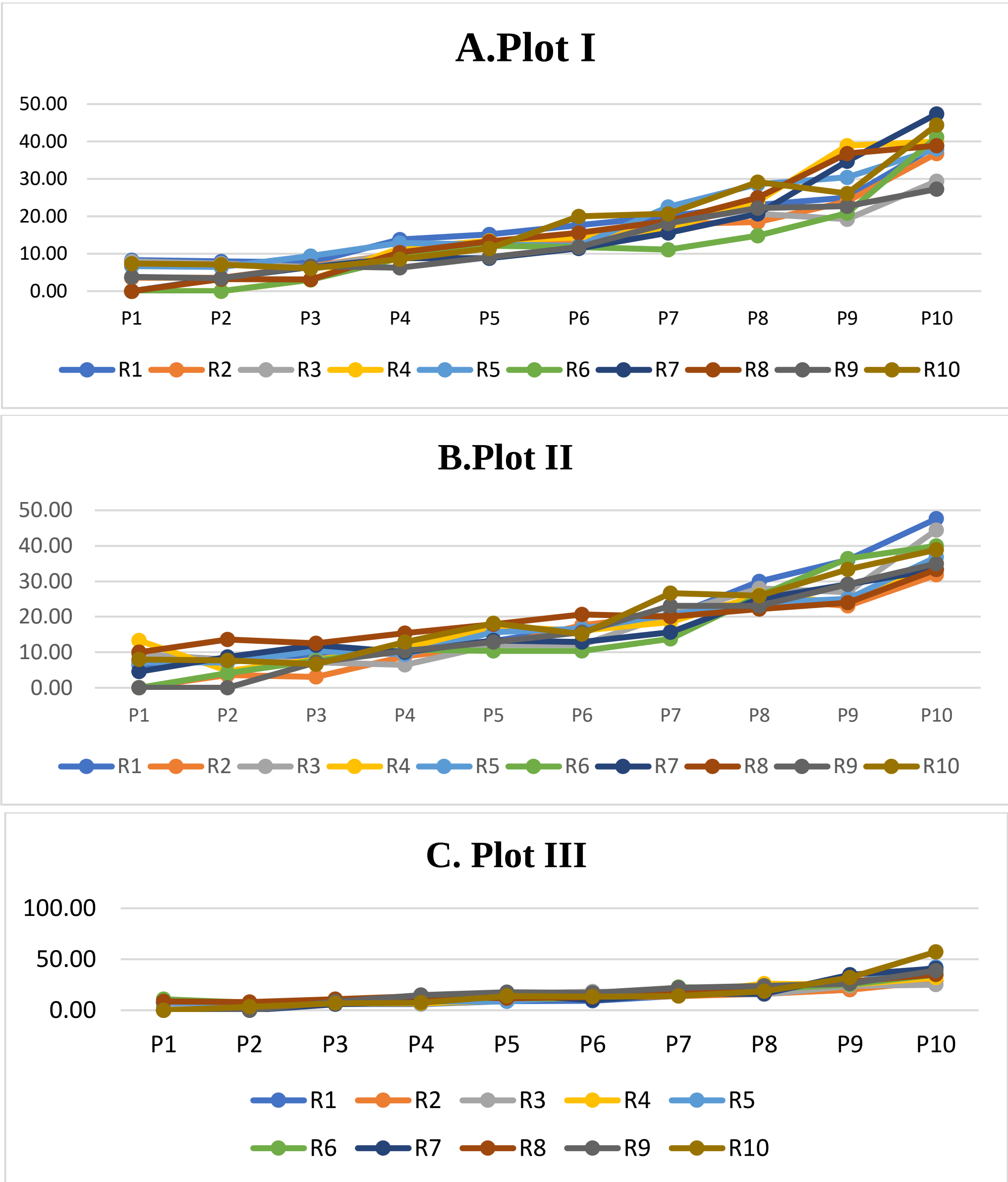
Bacterial leaf blight disease had 2 infectious phases, which were infection in the early vegetative phase which can cause crop failure, while in the generative phase it caused unfilled grain or empty grain, resulted yield losses reach 50% (Shen & Ronald, 2002). Based on the *Ditlin* report 2011, it was stated that the spread of BLB in Indonesia in 2010 reached 110,248 ha, 12 ha of them were crop failures/empty. The report stated that the highest BLB transmission occurred in West Java, 40,486 ha, Central Java 30,029 ha, East Java 23,504 ha, Banten 3,745 ha, and Southeast Sulawesi 2,678 ha.

The Disease Development / Incident of Bacterial Leaf Blight Disease

The percentage of bacterial leaf blight disease was observed in 3 plots rice field that had been marked with a stake. The observations of the disease percentage were conducted every week on rice plants started from 28 days after planting. The percentage of leaf blight disease in the first plot in Subak Munggu (Picture 3a) from the first observation (28 days after planting) to before harvest (104 days after planting), almost all clumps experienced an increase in BLB symptoms every week, except in some clumps there was a decrease at several weeks of observation. The percentage of disease in the fifth clump decreased from 6,67% at 28 days after planting to 6,45% at 33 days after planting, this was due to the growth of healthy tillers, resulted a decrease in the percentage of disease calculation. The highest average percentage of leaf blight disease occurred at the end of the observation (104 days after planting) of 38,24%. In the tenth observation the seventh clump showed the highest percentage of disease, which was 47,37%, while the lowest percentage of disease was in the ninth clump of 27,27%.

The percentage of leaf blight disease in the second plot in Subak Munggu (Picture 3b) from the first observation (28 days after planting) to before harvest (104 days after planting), almost all of the clumps increased every week, except for some clumps, which experienced a decrease in several weeks of observation. For example, the percentage of disease in the fourth clump decreased from 13,33% at 28 days after planting to 4,76% at 33 days after planting, this was due to the growth of healthy tillers, resulted a decrease in the percentage of disease calculation. The highest average percentage of leaf blight disease occurred at the end of the observation (104 days after planting) of 37,63%. In the tenth observation of the first clump, the highest percentage of disease was 47,62%, while the lowest percentage of disease was in the second clump of 31,82%.

The percentage of leaf blight disease in the third plot in Subak Munggu (Picture 3c) from the first observation (28 days after planting) to before harvest (104 days after planting), almost all of the clumps increased every week, except for a few clumps which experienced a decrease in several weeks of observation. For example, the percentage of disease in the sixth clump decreased from 10,71% at 28 days after planting to 7,41% at 33 days after planting and 6,67% at 41 days after planting, this was due to the growth of healthy tillers, resulted a decrease in the percentage of disease calculation. The highest average percentage of leaf blight disease occurred at the end of the observation (104 days after planting) of 36,94%. In the tenth observation, the tenth clump showed the highest percentage of disease, which was 57,14%, while the lowest percentage of disease was in the third clump of 25%.



Picture 3. The percentage of Bacterial Leaf Blight Disease in Subak Munggu plot I, II, III. (P1 (28 days after planting (DAT)), P2 (33 DAT), P3 (41 DAT), P4 (51 DAT), P5 (64 DAT), P6 (71 DAT), P7 (79 DAT), P8 (88 DAT), P9 (95 DAT), P10 (104 DAT). R1-R10 indicated clumps of rice plants.

The disease development in an area was highly dependent on the concept of disease incidence which was influenced by host plants, pathogens, and environmental factors that known as the disease triangle concept which was

(Adinugroho, 2008). The environment, especially the abiotic environment, which very influential on the disease development was temperature, humidity and rainfall. Temperature, humidity, and rainfall at the time of observation of disease development in Subak Munggu have been analyzed; the data that used were sourced from World Weather Online and Rice UPT BBU Badung Regency 2019. Based on rainfall data in the first week of February was 3 mm and until the third week 22 mm. The increased in rainfall affected the attacks percentage on each plot. The increase in rainfall result an increase in the percentage of disease in the first week to the third, but in some clumps there was a decrease in the percentage of disease. The decrease in the disease percentage was caused by the emergence of healthy tillers in several clumps, resulted a decrease in the percentage of disease calculation. In the third week, the percentage of disease increased due to high rainfall reaching 22 mm.

In March, in the fifth week, the rainfall reached 18 mm and in the sixth week of 14 mm rainfall. This rainfall resulted in a greater increase in the percentage of disease in each clump; while in April, the observations in the tenth week, there was only 1mm of rainfall but in the seventh to tenth week, the rice plants had decreased in tillers caused by the age factor of the rice plant. Therefore, all of the clumps in the plots experienced an increase in the percentage of disease caused by rice tillers started to decrease and disease also increased in each clump or sample and rainfall, temperature and humidity could also affect the disease development. The development of bacterial leaf blight disease was very influenced by the factors of rainfall, temperature, and high humidity because it spread very easily through rain and wind and outside the planting season, bacteria can live for 1-3 months depended on the humidity and the soil acidity and the temperature range for the colony growth which was 5-40°C, but the optimal temperature for growth was between 25-30°C. (OEPP/EPPO, 2007).

The Relation of Disease Percentage and Yields

Table 1 showed that the attack of bacterial leaf blight on rice plants in the first plot by looking at the number of tillers, clump weight and the percentage of attacks on the tenth observation that occurred in each clump affected the grain yield. In clump 7, the attack was very high the tenth observation, which was 47,37%, caused the grain yield to be lower than the other clumps, which was 65,24g. While in clump 9 which had a very low percentage of attack, was 27,27%, so it has a very high grain yield compared to other clumps, which was 81,71g. The weight results of the clump without grains were seen through the number of tillers, so that the clump with a high number of tillers will produce a high weight of the clump without grain, for example in the eighth clump and the ninth clump which had a high number of tillers, which was 22 tillers, so that obtained optimal weight of grain-free clumps were 121,46g and 124,43g. While the percentage of disease had more influence on grain weight because bacterial leaf blight disease was more identical in leaves of rice plants which caused inhibition of the photosynthesis process and inhibited the process of grain production of rice plants.

Table 1. Tillers observation result, disease percentage, weight of clump without grain, and grain weight on each clump of observation on plot I.

Clump	Tiller	Attack Percentage on the Tenth Observation (P10) (%)	Weight of Clump Without Grain (g)	Grain Weight (g)
R1	18	38,89	104,15	70,80
R2	19	36,84	109,68	74,72
R3	17	29,41	102,90	75,15
R4	20	40,00	114,53	73,73
R5	21	38,1	115,78	72,25
R6	17	41,18	98,48	68,19
R7	19	47,37	104,44	65,24
R8	22	38,89	121,46	73,78
R9	22	27,27	124,43	81,71
R10	18	44,44	97,26	66,67
Average	**19,30**	**38,24**	**109,31**	**72,22**

Where is the data source from (personal data resulted from research)

Table 2 showed that the attacks of bacterial leaf blight on rice plants in the second plot by looking at the number of tillers, clump weight and the attacks percentage on the tenth observation that occurred in each clump affected the grain yield in each clump. In clump 1, it can be seen that the attack was very high at the tenth observation, which was 47,62%, causing a very low grain yield of 68,47g. While in clump 2 which has a very low percentage of attack, which was 31,82%, so it has a very high grain yield compared to other clumps, in which 76,69g. The weight results of the clump without grain were seen through the number of tillers, thus the clump with a high number of tillers will produce a high weight of the clump without grain, for example in the second clump which has a high number of tillers, which was 22 tillers, therefore the weight results of the clump without grain were optimal, which was 117,56g. While the percentage of disease has more influence on grain weight because bacterial leaf blight disease was more identical in leaves of rice plants which caused inhibition of the photosynthesis process and inhibited the production process of rice plants grain.

Table 2. The observation result of tillers, disease percentage, weight of clump without grain, and grain weight in each observation clump in plot II.

Clump	Tiller	Attack Percentage on the Tenth Observation (P10) (%)	Weight of Clump Without Grain (g)	Grain Weight (g)
R1	21	47,62	112,82	68,47
R2	22	31,82	117,56	76,69
R3	18	44,44	95,89	67,58
R4	20	35,00	110,22	72,47
R5	19	36,84	107,23	71,29
R6	20	40,00	107,73	70,88
R7	21	33,33	115,5	74,51
R8	21	33,33	113,71	73,77
R9	20	35,00	112,79	73,40
R10	18	38,89	103,22	70,63
Average	**20,00**	**37,63**	**109,67**	**71,67**

Where is the data source from (personal data resulted from research)

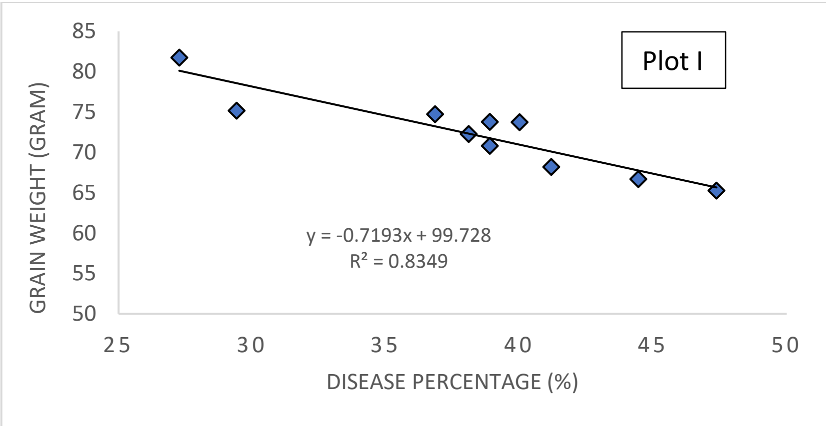
Table 3 showed that the attack of bacterial leaf blight on rice plants in the third plot by looking at the number of tillers, clump weight and attacks percentage on the tenth observation that occurred in each clump affected the grain yield in each clump. In clump 10, it can be seen that the attack was very high at the tenth observation, which was 57,14%, causing the grain yield to be very low compared to other clumps, in which 57,65g. While in clump 3 which has a very low percentage of attack, which was 25%, therefore it has a very high grain yield compared to other clumps, which was 86,6g. The weight results of the clump without grains were seen through the number of tillers, so that the clump that has a high number of tillers will produce a high weight of the clump without grain, for example in the third clump which has a high number of tillers, which was 24 tillers, thus get the optimal weight of the clump without grain, which was 127,31g. While the percentage of disease has more influence on grain weight because bacterial leaf blight disease was more identical in leaves of rice plants which caused inhibition of the photosynthesis process and inhibited the production process of rice plants grain.

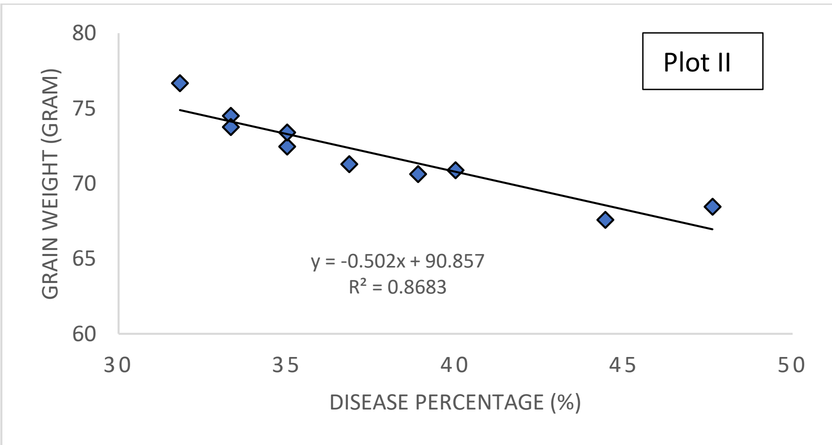
Table 3. The observation result of tillers, disease percentage, weight of clumpwithout grain, and grain weight in each observation clump in plot III.

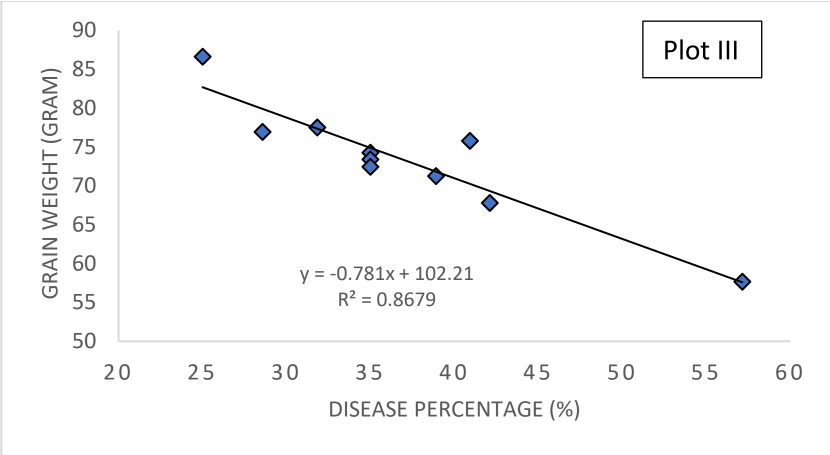
Clump	Tiller	Attack Percentage on the Tenth Observation (P10) (%)	Weight of Clump Without Grain (g)	Grain Weight (g)
R1	20	35,00	112,18	74,24

R2	21	28,57	119,54	76,95
R3	24	25,00	127,31	86,60
R4	22	31,82	121,88	77,51
R5	19	42,11	107,72	67,79
R6	20	35,00	110,03	73,40
R7	22	40,91	118,87	75,76
R8	20	35,00	108,2	72,46
R9	18	38,89	104,66	71,23
R10	14	57,14	91,32	57,65
Average	**20,00**	**36,94**	**112,17**	**73,36**

Where is the data source from (personal data resulted from research)







Picture 4. The relation of BLB disease attack percentage with the weight grain in plot I. II and III.

Table 4 showed that the disease percentage greatly affected the grain yield of rice plants due to the damage on the leaves, this damage caused the photosynthesis of rice leaves to be disrupted and the process of filling grain was not perfect, so that the grains became empty (Mew et al., 1982). The percentage of the attack of bacterial leaf blight on the leaves of rice plants can be measured by the level of leaf chlorophyll by using a chlorophyll meter. Leaf measurements started from the top of the leaf, the middle of the leaf and the base of the leaves of rice plants that were attacked by bacterial leaf blight. In the attack percentage, there were various CCI (Chlorophyll Content Index) on each leaf. Starting from the smallest attack, which was 10% - 20%, it obtained large leaf chlorophyll, which was at the top of the leaf 28,8CCI, the middle of the leaf 37,5CCI and at the base of the leaf 51,5CCI and an average of 39,3CCI. While in the percentage of 81% to empty, it obtained very small leaf chlorophyll, which was 12.2CCI at the top, 18,4CCI in the middle and 22CCI at the base of the leaves and an average of 17,5CCI. It can be concluded that the higher the percentage of the attacks of bacterial leaf blight that occurred on the leaves of rice plants, the lower the chlorophyll value in the leaves, causing inhibition of the photosynthesis process and the grain filling process on rice plants.

Table 4. The data of leaf chlorophyll based on the attack percentage

No	Bacterial Leaf Blight Attack Intensity (%)	Leaf Chlorophyll (CCI)			Average (CCI)
		Top	Middle	Base	
1	0	32,4	42,8	52	42,4
2	0 - 20	28,8	37,5	51,5	39,3
3	21 - 40	14,5	34,8	41,8	30,4
4	41 - 60	14	30,4	38,4	27,6
5	61 - 80	13,8	25,4	28	22,4
6	81 - Empty	12,2	18,4	22	17,5

Where is the data source from (personal data resulted from research)

Based on the results of this study, the development of BLB disease and the effect of damage to rice can be calculated. The results of this study were very important as a guide for pest observers (Department of Agriculture) for forecasting damage in one rice planting season. Thus, the possibility of damage and insurance claims to JASINDO (Agriculture Insurance Agency) can be predicted in one planting season. Direct observation (ground base method) was a method that is currently used for agricultural insurance purposes to support programs from the government (Ministry of Agriculture the Republic of Indonesia) (Ministry of Agriculture of The Republic of Indonesia, 2018; Surning et al., 2018; Ministry of Agriculture of The Republic of Indonesia, 2021)

CONCLUSION

Based on the results and discussion in the study, it can be concluded that: The development of bacterial leaf blight in Subak Munggu, Munggu Village, Mengwi Sub-district, Badung Regency, at ten weeks of observation showed an increase in disease development in all plots and every week of observation. A high percentage of disease attacks will cause low weight of rice grain that produced.

RECOMMENDATION

The method of assessing BLB damage which has been conducted by direct observation in the field (ground base method) needs to be developed using modern methods such as drone and remote sensing to support agricultural insurance.

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