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Determining the potential of sonic bloom in inducing rice resistance (*Oryza sativa*) against the attack of bacterial leaf blight disease caused by *Xanthomonas oryzae*

Abstract. *Xanthomonas oryzae* which causes bacterial leaf blight disease is one of the main pathogens that attack rice crops. The threat of losing rice yields due to *X. oryzae* needs to be avoided by implementing appropriate control strategies. Sonic bloom has the potential to induce plant immunity against diseases. Hence, this study aimed to examine the potential of sonic bloom in inducing rice resistance to bacterial leaf blight. The experiment was arranged in a Randomized Block Design (RBD) with five treatments and three replications. The treatments consisted of several frequencies, namely PR (0.5 - 1 kHz and inoculated with *X. oryzae*), PS (3 - 5 kHz and inoculated with *X. oryzae*), PT (7 - 10 kHz and inoculated with *X. oryzae*), KP (inoculated with *X. oryzae* without sonic bloom treatment), KN (no bacterial inoculation and sonic bloom treatment). The results showed that all sonic bloom treatments (LF, MF, HF) had a significant effect on increasing the intensity of BLB disease in rice plants. This showed that sonic bloom in this range does not have the potential to induce rice plant resistance to BLB. Further research to find out the causes of this is needed.

Keywords: Bacterial leaf blight • Cicadas • Disease intensity • *Xanthomonas oryzae*

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Introduction

Bacterial leaf blight (BLB) is one of the crucial diseases in rice cultivation. BLB disease caused by *Xanthomonas oryzae* pv. oryzae is one of the causes of rice yield loss with a percentage of 80-90% in rice-producing countries, especially in Asia (Vikal & Bhatia, 2017). According to the Badan Peramalan Organisme Pengganggu Tumbuhan (Center for Forecasting Plant Pest Organisms) Indonesia (2021), the distribution of BLB disease in Indonesia during the 2021 rice planting season was estimated to be 28,743 ha with the highest attack in West Java Province covering 6,381 ha.

The threat of loss of rice yields due to BLB needs to be avoided by implementing appropriate control strategies. In general, control can be carried out in various practices, including mechanical, physical, chemical, cultural and biological techniques (Suganda, 2020). Synthetic chemical pesticides or bactericides, such as nickel dimethyl dithiocarbamate, dithianon, and phenazine, are frequently used to manage BLB at the farm level (Kurniawati, 2021). However, utilizing chemical pesticides as the only form of control will have numerous detrimental implications on human health and the environment (Nicolopoulou-Stamati et al., 2016). Employing the resistant varieties is one of the practices in controlling BLB, it must be balanced with the application of other cultivation elements. Spacing, proper fertilization, and land sanitation are essential elements of healthy farming (Kurniawati, 2021). In light of this, integration of these practices should be promoted to get a great result, this concept is well-known as integrated pest management (IPM) (Deguine et al., 2021). A development of various control practices is important for supporting IPM.

Sonic bloom is a new control practice that can be developed for controlling BLB disease in rice. It is presumed that plant resistance can be increased by using sonic bloom by generating energy or vibration around the plant through the use of sound waves so that it can suppress attacks by pests and diseases. The research of Hassanien et al. (2014) shows that sonic bloom treatment could reduce spider mites, aphids, gray mold, leaf blight and viral diseases by 6%, 8%, 9%, 11%, and 8% respectively on tomatoes in the greenhouse. Furthermore, the use of sonic bloom can reduce the attack of *Rhizoctonia solani* which causes rice sheath blight by up to 50%. Hormones that can increase plant resistance to pests are produced by plant gene expression which is influenced by mechanical stimuli in the form of sonic bloom (Joshi et al., 2019). Sonic bloom has not been widely used by farmers in Indonesia. The use of sonic bloom technology in rice is expected to be able to induce plant resistance to BLB disease.

In addition, sonic bloom is a technology that can trigger the opening of stomata (Ai et al., 2021; Prasetyo & Wicaksono, 2019). The high sound frequency used through sonic bloom on plants can cause the stomata opening to become wider. This has implications in increasing the uptake of water, nutrients, and CO_2 through the stomata on the leaves thereby helping the optimization of the photosynthesis process. In addition, mustard plants that were treated with sonic bloom with a frequency of 3,000 Hz had a 20% higher weight yield compared to the control treatment (Putri et al., 2021).

The sound of cicadas is often considered a dominant and iconic feature of nature in many regions around the world. This characteristic buzzing or droning noise, produced by male cicadas to attract females, is particularly prominent during the hot summer months (Haskell, 2018). Anas & Kadarisman (2018) used cicadas sound as a sonic bloom technology for enhancing rice growth. Study on the effect of cicadas sound on rice resistance would be interesting.

The purpose of this study was to determine the potential of sonic bloom and its appropriate frequency level in inducing rice resistance to BLB disease. The novelty in this study is the utilization of sonic bloom with the sound of cicadas in inducing rice resistance to BLB disease.

Materials and Methods

Time and Location. This experiment was conducted for four months from June - September 2022 at the Sari Kedele Farm Greenhouse, Jatinangor and the Entomology and Biotechnology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran.

Materials. The equipment used in this study were Robot speakers "RB100" as a sound source with specifications of frequency response range 100 Hz - 18 kHz, ability to work for 8 hours, and can be used with Bluetooth, USB, or TF Card, flash drives, saws, scissors, rulers, knives, UV transparent plastic, wood, hammers, nails, chlorophyll meters, microscopes, digital scales, laboratory equipment (Bunsen burner, object glass and covers, reaction tubes, petri dish, spatula), Microsoft Excel and SmartstatXL Excel Add In, Adobe Photoshop CC 2022 software, ImageJ software, Audacity software, frequency meter application, sound meter application. The materials used were Mapan 05 variety rice seeds, buckets, topsoil of a paddy field, compost, manure, NPK 15-15-15 fertilizer, water, *X. oryzae* isolate, distilled water, and clear nail polish.

Experimental Design. The experiment was arranged in a Randomized Block Design (RBD) with five treatments and three replications. Sonic bloom was applied for 3 hours per day (07.00 - 10.00 WIB) with three different frequencies taken from the sound of the cicadas or in Indonesia it's called "tonggeret". The length of sonic bloom exposure time refers to the research of Hendrawan et al (2020), which stated that the 3-hour exposure time showed the best results on several variables of growth and physiology of kailan plants.

LF : 0.5 - 1 kHz and inoculated with *X. oryzae*
MF : 3 - 5 kHz and inoculated with *X. oryzae*
HF : 7 - 10 kHz and inoculated with *X. oryzae*
PC : inoculated with *X. oryzae* without sonic bloom treatment
NC : no bacterial inoculation and sonic bloom treatment

Notes: LF, Low Frequency Treatment; MF, Moderate Frequency Treatment; HF, High Frequency Treatment; PC, Positive Control; NC, Negative Control.

Sonic Bloom Chamber Construction. A wooden frame was constructed and coated with transparent UV plastic to form a chamber for rice growth. After that, the speakers were placed in it. The speaker was placed at the top of the device by hanging using a rope. This device was made to keep each treatment under the same conditions and facilitate observation. The chamber is presented in Figure 1.

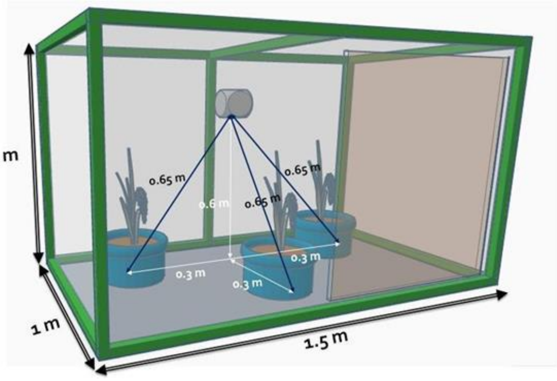

Figure 1. Design of sonic bloom chamber

The sound used was a high quality sound of cicadas obtained from the freesound.org website. The sound was adjusted and altered for frequency and intensity according to the desired range for this research using the Audacity software. The accuracy of the frequency was examined using a frequency meter application that had been tested for functionality (calibration) beforehand. Sound intensity was measured using the sound meter application.

Planting Media Preparation. Planting media was prepared by adding topsoil of paddy field and manure that had been mixed into a bucket with a ratio of 1 : 1. The mixture of planting media was loosened first and soaked before the rice was planted.

Rice Planting. Mapan 05 variety rice seeds were first sown in trays containing planting media for 15 days. Seedling that had been sown was transplanted into a bucket that had been prepared beforehand. The buckets were put into the sonic bloom chamber according to their respective treatments then stored in the greenhouse. Routine maintenance was carried out such as watering, weeding, and fertilization.

***X. oryzae* Inoculation.** *X. oryzae* was inoculated when the rice was 16 days after sowing (DAS). The inoculation technique used was spraying a suspension of *X. oryzae* with a density of 10^{10} CFU/mL as much as 5 mL by using a spray bottle on each rice plant in a bucket directly without being injured.

Observation. Observations were conducted on several variables included:

a. Disease parameters (disease intensity and development rate of the disease);
b. Physiological parameters (stomatal opening, chlorophyll index, and stomatal conductance);

c. Growth parameters (plant height, fresh weight, root length, leaf area, number of tillers, and number of leaves).

Observations of disease intensity were made daily for 14 days by scoring each treatment pot, then calculated using a formula:

$$DI\ (\%) = \sum (n \times z)/(N \times Z) \times 100\%$$

With n = number of samples that have the same score value; z = score value; N = total number of samples observed; and Z = highest score used. Observations of the development rate of disease progression were seen from the graph of intensity development, infection rate (r) values, Area Under Disease Progressive Curve (AUDPC) values, and the percentage of disease inhibition.

For growth parameters and stomatal opening, observations were made on day 14 shortly after the plants were harvested. Then for physiological parameters except stomatal opening, observations were made every 3 days for 14 days.

Data analysis. Data analysis was performed using Excel Software and SmartstatXL Excel Add-In. Analysis data was conducted using Analysis of Variance (Anova) for all observation variables. If Anova showed a significant effect, then Duncan's multiple range test (DMRT) was conducted at the 5% significant level.

Result and Discussion

Disease Intensity. Based on Table 1, the intensity of leaf blight in LF, MF, and HF treatments is significantly different from NC and PC. The sonic bloom treatment has a higher disease intensity when compared to both controls. In addition, NC and PC had the lowest AUDPC values, which amounted to 30.21 and 58.92 and were significant to the sonic bloom treatment. LF had an AUDPC value of 138.41 and was significant against MF and HF which had AUDPC values of 189.03 and 180.14. The higher AUDPC value in the sonic bloom treatment indicates that the development of disease severity in rice plants treated with sonic bloom is higher than both controls.

Disease progression from the first day to 14th day after inoculation showed an increase in all treatments (Figure 2). On day 3, HF showed a significant change in the graph of disease intensity. LF and MF showed significant changes on day 4, while PC showed significant changes on day 12. This is thought to be because *Xoo* has passed the lag phase faster in the sonic bloom treatment compared to control.

Table 1. Disease intensity and AUDPC

Treatment	Disease Intensity (%)	AUDPC
NC	2.12 a	30.21 a
PC	4.99 b	58.92 a
LF	9.18 c	138.41 b
MF	10.10 c	189.03 c
HF	9.24 c	180.14 c

Note: *Numbers followed by the same letter in the same column are not significantly different based on Duncan's test at 5% significance level. NC, Negative Control; PC, Positive Control; LF, Low Frequency Treatment; MF, Moderate Frequency Treatment; HF, High Frequency Treatment; DI, Disease Intensity; AUDPC, *Area Under the Disease Progress Curve*.

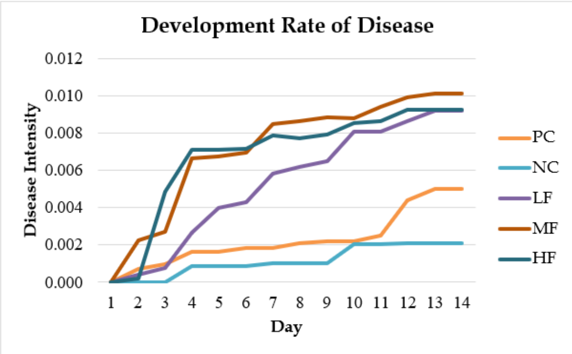

Figure 2. Developmental rate of BLB disease with several sonic bloom treatments.

The high values of disease intensity and AUDPC in LF, MF, and HF are thought to be due to sound exposure that not only affects plants, but can also affect bacteria. Juergensmeyer (2004) stated that the lag phase of bacteria can be shortened and the growth rate of bacterial cells can increase due to the influence of sound frequency. In addition, sound vibration can also provide optimum conditions for bacterial cell membranes to increase metabolic activity and bacterial growth (Shaobin et al., 2010; Ying, 2009).

Shah et al. (2016) stated that the treatment of raag ahir bhairav music exposure with a frequency range of 150 - 7811 Hz can increase the growth and production of exopolysaccharide (EPS) in *Xanthomonas campestris* by 6.66% and 32.06%, respectively. Bianco et al. (2016), stated that Xanthomonas spp. produces the main EPS in the form of xanthan which has a function as a bacterial defense from stress and helps penetration on the plant surface with the formation of biofilms.

Sonic bloom treatment in this study was able to significantly increase stomatal opening against the control. It is also thought to cause BLB disease intensity in the sonic bloom treatment to be higher when compared to the control. Sawinski et al. (2013), mentioned that bacteria cannot penetrate directly into the leaf epidermis so that endophyte colonization occurs through natural holes, such as stomata and hydathodes or through wounds. Stomatal pores are limited by two guard cells that regulate gas exchange and transpiration through changes in their openings in response to the environment, such as effective photosynthesis lighting, CO2 levels, water availability, and the stress hormone abscisic acid (ABA) (Sirichandra et al., 2009). Stomatal closure is one of the plant defense responses in the face of pathogen attack (Nejat & Mantri, 2017).

Effect of Sonic Bloom Treatment with Different Frequency on Physiological Characteristic. Based on statistical analysis, sonic bloom treatment showed a significant effect on stomatal opening, but not significant on chlorophyll index and stomatal conductance. Figure 3a shows that LF, MF, and HF are significantly different from NC and PC in the variable of stomatal opening. LF, MF, and HF were not significantly different and had mean values of stomatal opening of 6.55 µm, 7.18 µm, and 6.35 µm, respectively. This is in accordance with the principle of the ability of sound waves in sonic bloom to trigger stomatal opening by generating energy or vibration around the plant (Ai et al., 2021; Prasetyo & Wicaksono, 2019).

Chlorophyll functions as a catcher and converter of sunlight energy into chemical energy used by plants for photosynthesis (Melkozernov & Blankenship, 2006). Based on Figure 3b, sonic bloom treatment did not show a significantly different effect on chlorophyll index. This is contrary to the research of Hendrawan et al. (2020), which states that sonic bloom treatment can increase the photosynthesis process optimally, which also has an impact on increasing the amount of leaf chlorophyll.

Sonic bloom treatment did not show a significant effect on stomatal conductance (Figure 3c). Stomatal conductance is a representation of the number of stomata that open in response to the environment. The higher the stomatal conductance, the greater the number of open stomata. The greater number of open stomata implies an increase in CO_2 fixation so that the photosynthetic rate of plants will increase and growth will be higher (Nuraisah et al., 2019).

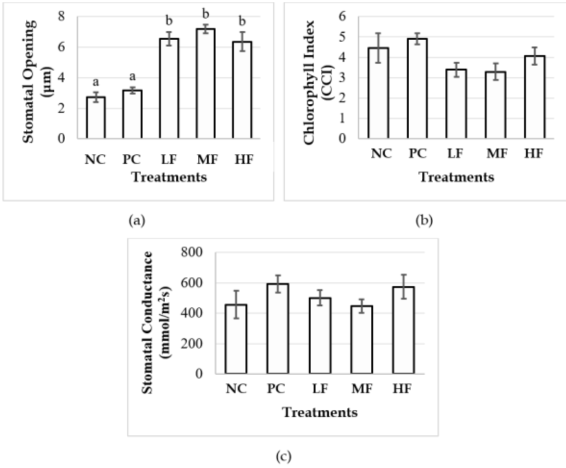

Figure 3. Effect of Sonic Bloom Treatment with Different Frequencies on the Physiological Characteristic (a) stomatal opening; (b) chlorophyll index; (c) stomatal conductance; NC, Negative Control; PC, Positive Control; LF, Low Frequency; MF, Medium Frequency; HF, High Frequency

Effect of Sonic Bloom Treatment with Different Frequency on Plant Growth. Based on statistical analysis, sonic bloom treatment showed a significant effect on plant height, number of leaves, and fresh weight. However, it did not show a significant effect on root length, leaf area, and number of tillers. Based on Figure 4a, further test analysis shows that MF and HF have lower mean values and are significant to both controls in the plant height variable, but LF does not show a significant value to both controls.

Based on Figure 4b, sonic bloom treatment does not show a significant effect on PC on the number of leaves variable, but it is significant for NC. NC has the highest mean value, which is 42.61. Similar to the fresh weight variable in Figure 4c, NC has the highest mean value, which is 16.46 g and significant with MF and HF. Han et al. (2008), stated that Xoo infects plants through natural openings such as hydathodes and/or injured plant parts, then colonizes the xylem. This causes blockage of the xylem and disruption of nutrient and water transport from the roots, resulting in suboptimal plant growth.

Roots are one of the important components in supporting the growth of rice plants that have a role in the absorption of water and nutrients (Sumadji & Purbasari, 2018). Based on Figure 4d, the analysis results show that the sonic bloom treatment does not show a significant effect on the root length variable. Similarly, the variables of leaf area and number of tillers can be seen in Figure 4e and 4f. Bhandawat & Jayaswall (2022), stated that plants are naturally sensitive to sound perception, but the response shown by plants is slow and much more subtle, unlike animals or humans who can express their response quickly.

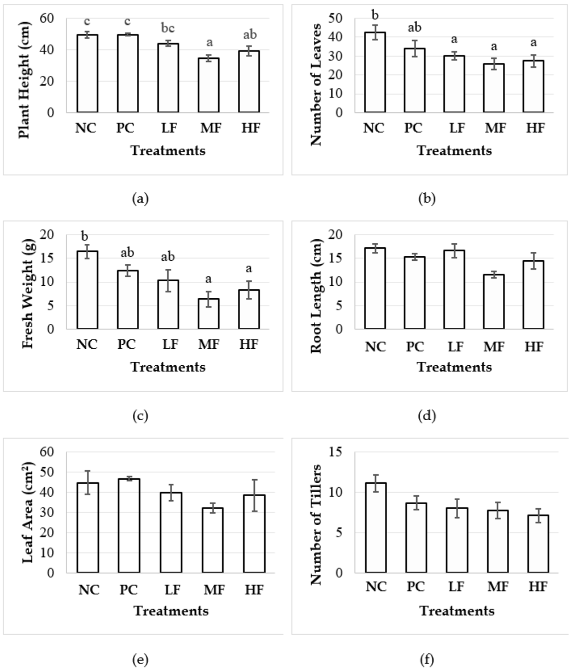

Figure 4. Effect of Sonic Bloom Treatment with Different Frequencies on Plant Growth (a) plant height; (b) number of leaves; (c) fresh weight; (d) root length; (e) leaf area; (f) number of tillers; NC, Negative Control; PC, Positive Control; LF, Low Frequency; MF, Medium Frequency; HF, High Frequency

Conclusion

Sonic bloom with frequency ranges of 0.5 - 1 kHz (LF), 3 - 5 kHz (MF), and 7 - 10 kHz (HF) has not shown potential in inducing rice resistance to BLB disease. Further experiments are needed to further examine the reasons for the inability of sonic bloom to induce rice resistance to BLB disease and explore the frequency range that has the potential to induce rice resistance to BLB disease.

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