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Plant acoustic frequency technology control system to increase vegetative growth in red-lettuce

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ABSTRACT

The application of plant acoustic frequency technology (PAFT) can spur plant growth, increase productivity, immune system, quality, and extend the shelf life of agricultural products after harvest. The application of sound waves in plants can stimulate the opening of the stomata to optimally absorb nutrients and water. This study aims to determine the effect of frequency and time of PAFT exposure by utilizing Javanese gamelan traditional music on the vegetative growth of red-lettuce (Lactuca sativa var.). Javanese gamelan music used was titled Puspawarna with variations in the frequency of 3-5, 7-9, and 11-13 kHz. The variation of exposure time of sound waves was 1, 2, and 3 hours. PAFT exposure was given routinely in the morning and evening. The results of this study indicated that the best treatment was at 3-5 kHz with an exposure time of 2 hours. This treatment gave a significantly better effect when compared to plants without PAFT. The best combination of frequency and time of PAFT exposure produced 10 leaves, plant height of 9.4 cm, wet weight of 4 g, dry weight of 0.22 g, leaf area of 27.19 cm^2, leaf red mean of 63, and stomata opening width of 145.44~206.59 µm.

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1. INTRODUCTION

Agriculture at present experiences various problems such as climate change due to global warming, the use of chemicals damaging the environment, ineffective use of energy, increasing the human population, and reduced fertile land due to competing with land for housing [1]. To overcome this problem, it is necessary to have a flexible agricultural system without depending on the availability of land, climate and weather conditions, energy use, agricultural materials, and most importantly producing agricultural products effectively and productively. One technology that can overcome these agricultural problems is the closed bio-production system [2, 3], a technology to increase crop productivity by manipulating environmental factors such as water, nutrition, temperature, humidity, CO2, and light [4-7]. However, more research is required to discuss the effect of sound waves on crop productivity and growth in a closed bio-production system. Naturally, in an open environment, plant growth is also indirectly affected by the surrounding sounds, one of which is animal sound and wind [8-11]. Plants treated with acoustic frequency technology (PAFT) can make adjustments at molecular/physiological and morphological levels. Furthermore, when species and plant tissues are different, they have responses to different PAFT frequencies [12]. In several studies, the utilization of PAFT has been proven to be successful in increasing crop productivity [13-16]. Sound waves can stimulate endogenous hormones, the optimal opening of leaf stomata, improving the effectiveness of plant growth [17, 18]. Studied conducted by Creath and Schwartz [19] indicated that PAFT with music sounds can significantly increase crop productivity and quality compared to conventionally cultivated plants without PAFT. Audible sound control at a frequency of 2200 Hz has indicated to significantly increase the productivity of microalgae Picochlorum oklahomensis by 27.5% [20, 21]. Exposure with PAFT for 8 hours per day for 22 days is believed to increase the effectiveness of microalgae (H. pluvialis) growth by 58% which is higher than microalgae without PAFT [22]. Sound frequency in the range of 0.1-1 kHz with exposure for 3 hours can increase the productivity of lettuce, spinach, cotton, rice, and wheat plants by 19.6, 22.7, 11.4, 5.7, and 17.0%, respectively. PAFT application has also been proven to increase shelf life and fruit quality after harvest [23]. Meng [24] proved that PAFT was able to significantly increase the effectiveness of the photosynthesis process and reduce the use of fertilizers in plants. PAFT has also been shown to be effective in stimulating vegetative growth and productivity of various plant species, such as Cucurbita pepo [25]; Gerbera jamesonii [26], Oryza sativa [27], Dendrobium officinale [28], Aloe arborescens [29], and Corylus avellana [30], which have proven to increase germination index, shoot height, plant fresh weight, and root system productivity. Qi [31] also proved in his research that PAFT is also useful for increasing disease resistance in plants.

Lettuce is a popular vegetable because it has many health benefits [32]. One type of lettuce plant is red lettuce (Lactuca sativa) which has red colored leaves. The red color of lettuce is caused by its high anthocyanin content which is one of the main compounds found in red lettuce [33]. Lettuce is widely used in various types of food preparations, increasing the need for lettuce plants, especially red lettuce which has a much higher nutritional content. High anthocyanin content in red lettuce can add higher nutritional and antioxidant values than in green lettuce [34-36]. In the study of Becker and Klaring [37], control of environmental parameters in CO2 was proven to increase phenol content and productivity of red lettuce. Light control settings in the study of Kawasaki [38] can increase polyphenol content and productivity of red lettuce plants. However, the production of red lettuce plants is still mostly conducted in conventional hydroponics [39]. Unfortunately, research conducted with environmental parameter control has been limited, specifically with PAFT application which has been thought to increase the productivity of red lettuce in the closed bio-production system.

The purpose of this study was to examine the effectiveness of PAFT on the vegetative growth of red-lettuce plants. The sound utilized as PAFT is the sound of traditional Javanese music of gamelan with the title Puspawarna [40]. In previous studies, it has been proven that Javanese gamelan music is effective compared to other types of music such as rock and jazz [41]. The treatments in this study include variations in frequency level and exposure time.

2. RESEARCH METHOD

This research was conducted in the Mechatronic Laboratory of Agroindustrial Tools and Machines, Department of Agricultural Engineering, Faculty of Agricultural Technology, Brawijaya University, Malang. Tools used in this study included: 1) The closed chamber in green house as a place for cultivation of red-lettuce; 2) Speaker Advance duo 050, as a sound source; 3) RTC DS3231, used to control the timing of voting; 4) Relay 4 channel 5 volts, to control the speaker automatically; 5) AC to DC 12 V10 A Power Supply, as an electronic circuit of power source; 6) Arduino uno atmega, as a microcontroller to run automation; 7) MP3 Decoder 5 volt (mono speaker), functions as a sound generator; 8) Sound level meter (Extech Instruments), to measure sound levels; 9) Hygrometer (Anymetre model: TH108), to measure air humidity levels; 10) Thermometer (Anymetre model: TH108), to measure the temperature level of the observed environment; 11) Lux Meter (Taxi 8720), to measure the intensity of environmental light; 12) Image analysis software application, to measure red leaf color index; 13) Digital Scales (I-2000) and (Camry), to measure wet weight of plants; 14) Olympus CX43 Binocular Microscope, to observe the width of the stomata opening. Materials used in this study included: 1) red-lettuce seeds, as the main object of treatment; 2) vermicompost fertilizer, as soil organic matter and nutrients for plants; 3) organic liquid fertilizer, to provide plant nutrition during growth; 4) nail polish, as the stomata printing medium of the leaf.

This study utilized several growth chambers with a size of 150×100 cm placed in a greenhouse. Each growth chamber had two speakers placed in the upper center of the growth chamber as presented in Figure 1. The speakers in the growth chamber were arranged downwards facing the plants at a distance of 60 cm. The plants used in this study were red-lettuce plants which were 14 days after seedling. Furthermore, red-lettuce plants were given PAFT treatment for 21 days in the morning at 07.00-10.00 and evening at 14.00-17.00. The sound used as PAFT treatment came from traditional Javanese Gamelan music with the title Puspawarna. The frequency produced from Javanese Gamelan music had been set and adjusted to the treatment by using the Audacity application. The measurement results of each frequency level were presented in Figure 2.

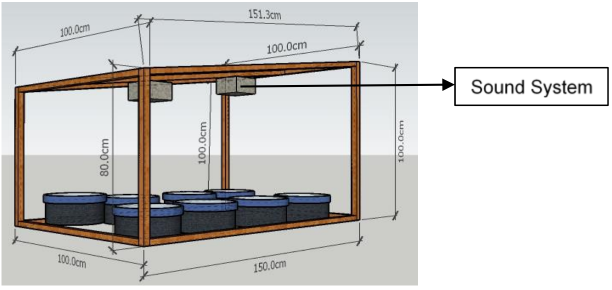
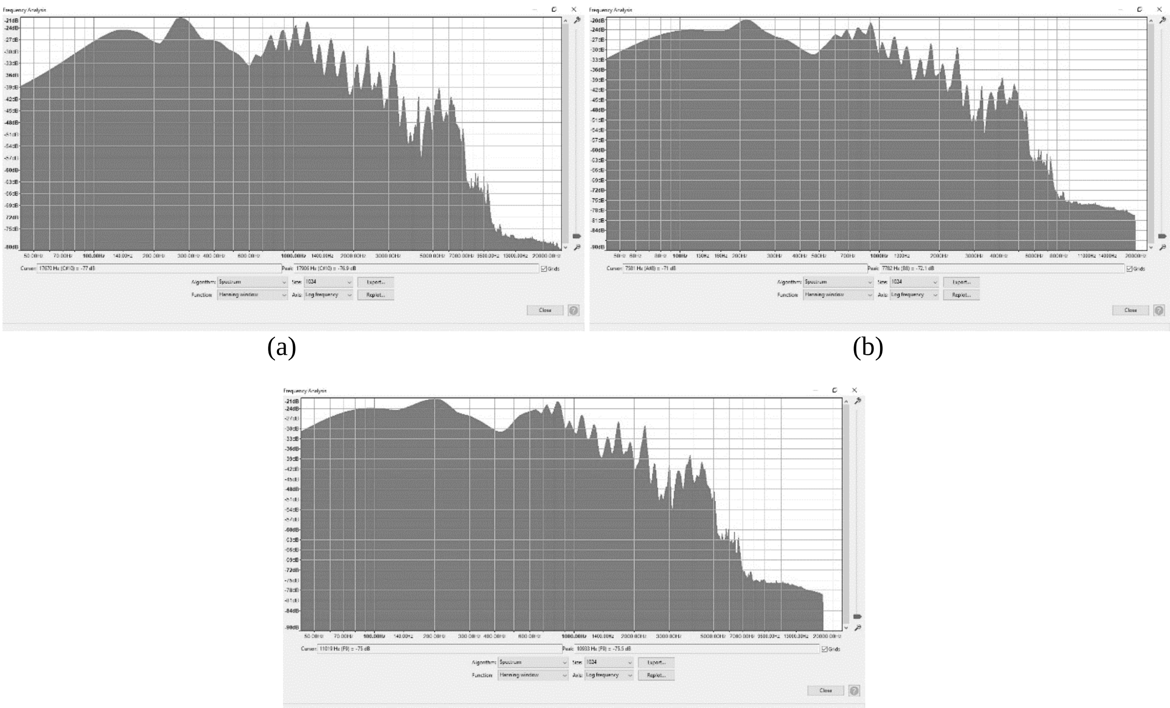


Figure 1. PAFT design in a red-lettuce cultivation chamber



(a) (b)

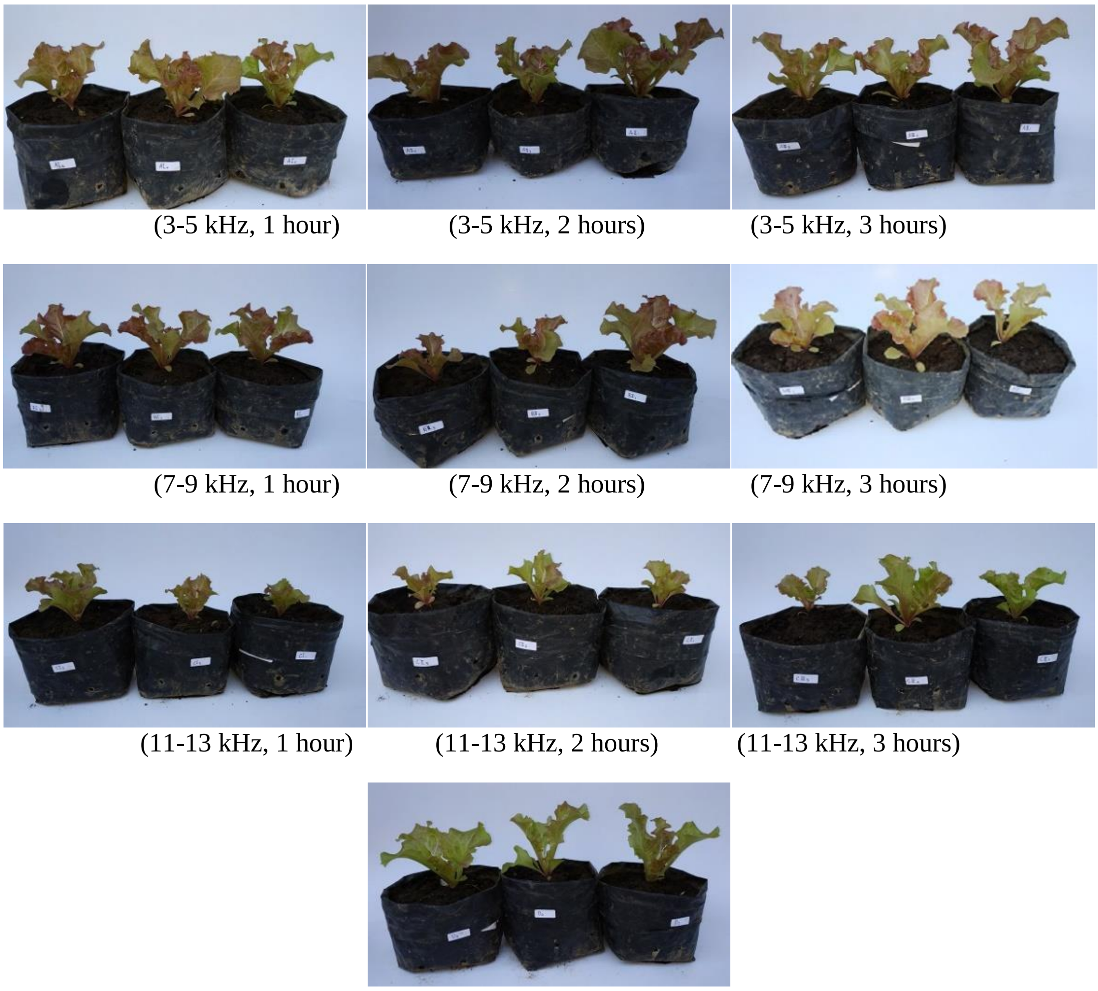
(c)

Figure 2. Frequency settings by using Audacity: (a) 3-5 kHz; (b) 7-9 kHz; (c) 11-13 kHz

Determination of the magnitude of the frequency and time of exposure in the study was based on preliminary research with variations in the frequency of 3-5, 7-9, and 11-13 kHz, with variations in exposure time for 1, 2, and 3 hours. The sound intensity level at each treatment was similar in the range of 68~76 db. Control plants that did not get PAFT treatment were used as a comparison with plants that received PAFT treatment. To optimize the growth of red-lettuce plants, environmental conditions had been conditioned optimally and uniformly for all treatments. Some of the environmental parameters included ambient temperature, air humidity, sound intensity (noise), water, nutrition, and sunlight intensity. PAFT testing on red-lettuce included observations on several parameters, such as the number of leaves (strands), plant height (cm), leaf area (cm^2), plant wet weight (g), plant dry weight (g), red mean leaves, and width stomata opening. Measurement of the number of leaves was conducted by counting the number of each leaf in the plant. Selected leaves were from leaves that had begun to open wide. Plant height measurements were measured from the base of the stem which was parallel to the surface of the growing media to the top shoots. The leaf area of the plant was measured by using the gravimetric method. Wet weight was measured by using a digital scale. Dry weight was measured after measuring the wet weight of the plant. Red-lettuce plants (wet weight) were then dried by using an oven at 70 ºC until a dry or constant plant weight was obtained. Measurement of red mean leaves with values ranging from 0~255 on red-lettuce leaves was required to determine the level of redness as characteristic of red-lettuce leaves. Red mean measurements by using image analysis software were based on Visual Basic 6.0. The width of the stomata opening was analyzed by using a digital microscope using a magnification of 40×.

3. RESULTS AND ANALYSIS

The results of the red-lettuce products that received PAFT treatment and those without treatment (control) were depicted in Figure 3. If visually noted, there were differences in the red-lettuce plants that received PAFT treatment with plants without treatment. Red-lettuce with the treatment looked better with bigger leaves and a more red color compared to untreated plants. In qualitative observations, PAFT gave more optimal results on vegetative growth of plants when compared to plants without PAFT (control).



(3-5 kHz, 1 hour) (3-5 kHz, 2 hours) (3-5 kHz, 3 hours)

(7-9 kHz, 1 hour) (7-9 kHz, 2 hours) (7-9 kHz, 3 hours)

(11-13 kHz, 1 hour) (11-13 kHz, 2 hours) (11-13 kHz, 3 hours)

(control)

Figure 3. Results of red-lettuce growth with PAFT and without PAFT

From the observation of the mean red leaf value in Figure 4, it was apparent that the highest average value was in the treatment of 7-9 kHz with 1-hour time which was equal to 68. Meanwhile, the lowest red mean observation results were in the 3-5 kHz treatment with 2 hours which was equal to 63. The higher red mean value of leaves caused brighter red color; while the lower red mean value of the leaves caused the redness to get closer to dark red. A more concentrated level of redness caused better red-lettuce vegetative growth. There was an interesting pattern in the PAFT exposure time parameter of 2 hours, where a more frequency level increased the red mean value of the leaf. The magnitude of the red mean on the leaves was not completely affected by PAFT exposure, because the results indicated an insignificant difference between plants with PAFT treatment and plants without PAFT. In this case, the concentration of anthocyanin in the red color of red-lettuce plants was more influenced by genetics, temperature, and light [42].

In terms of wet weight parameters of the plant, it was found that the highest average value of the treatment was in the 3-5 kHz treatment and the exposure time was 2 hours with a wet weight of 4 g. However, the lowest wet weight monitoring results were found in the treatment of 11-13 kHz with 2 hours of exposure time with a wet weight of 1.03 g. The graph of the observation for the wet weight of the red-lettuce plant with the average replicated data between each treatment was depicted in Figure 5. Figure 5 indicated an almost uniform pattern in PAFT with 2- and 3-hour exposure time parameters, where there was a tendency to increase the frequency level of PAFT, followed by decreasing wet weight of the plant. An interesting pattern was also depicted in the 7-9 kHz PAFT frequency level where the tendency of plant wet weight decreased along with longer exposure time at that frequency. ANOVA statistical test was conducted by using a real level (α = 0.05) in which it was found that the treatment with frequency variation had a significant effect on plant wet weight; while the exposure time had an insignificant effect on wet weight of red-lettuce plants. From the results of further tests of DMRT with a 5% significance level, the results indicated that treatment of frequency variations with the most significant effect was at 3-5 kHz frequency. From these results, it was apparent that PAFT had a significant influence on the wet weight of red-lettuce plants where the best treatment was at 3-5 kHz frequency with an exposure time of 2 hours. Wet weight or commonly called plant fresh weight was the initial weight of the plant after harvesting and the condition had not withered to find out plant biomass. According to Huang [43], the process of photosynthesis produced a biochemical reaction called plant biomass. In the research of Hassanien [44], the treatment of giving sound frequencies to plants can increase a better wet weight of plants compared to plants without PAFT treatment. In his research, the increase in the wet weight of plants can reach up to 25.63% greater when compared to plants without PAFT.

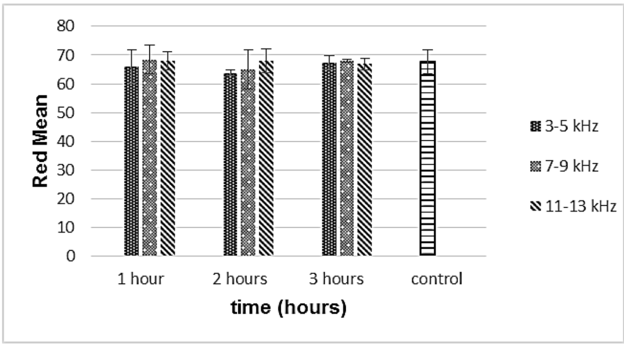


Figure 4. Relationship between the frequency variation and PAFT exposure time to the red mean value of red-lettuce leaves

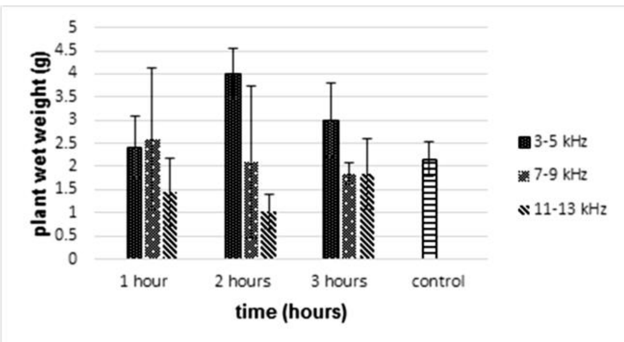


Figure 5. Relationship between variations in frequency and time of PAFT exposure to the wet weight of red-lettuce plants

The results of plant dry weight indicated that the highest average value of the treatment was at 3-5 kHz frequency with 2 hours exposure time and with a dry weight of 0.22 g. Meanwhile, the lowest value was on the frequency of 11-13 kHz and 2 hours of exposure time with a dry weight of 0.04 g. The graph depicting the results of dry weight observation of red-lettuce plants with average replication data between each treatment was presented in Figure 6. The graph pattern on plant dry weight parameters was generally similar to the wet weight parameter graph pattern. In terms of exposure time of PAFT 2 hours, dry weight pattern decreased along with increasing frequency level of PAFT given to plants. At the frequency parameter of 7-9 kHz, the result indicated a similar pattern between wet weight and dry weight of plants; when more exposure time was added, the dry weight of plants decreases. From the ANOVA statistical test results conducted by using a real level ($\alpha = 0.05$), it was found that the treatment with frequency variations had a significant effect, while the exposure time had no significant effect on red-lettuce plants. From the results of further tests of DMRT with a 5% significance level, the results indicated that the treatment of frequency variations had the most significant effect at 3-5 kHz frequency. From these results, it was concluded that the treatment of PAFT can have a significant effect on the dry weight of red-lettuce with the best treatment at a frequency of 3-5 kHz and exposure time of 2 hours. The results of this study were in line with several studies by Hassanien [44], where the dry weight of plants given PAFT treatment indicated an increase up to 58.73% compared to plant without PAFT.

The leaf area parameter became one of the important measurement factors in determining the vegetative growth of plants. From the observation data of leaf area parameters, it was revealed that the highest average value was in the treatment frequency of 3-5 kHz and the exposure time of 2 hours with a leaf area value of 27.19 cm^2. The lowest leaf area values were in the treatment frequency of 11-13 kHz and the exposure time was 2 hours with a leaf area value of 6.21 cm^2. The results of observing leaf area with various variations of PAFT treatment can be seen in Figure 7. The graph which presented the relationship between PAFT treatment and leaf area indicated a similar pattern in each PAFT exposure time parameter, where the plant leaf area decreased along with the increasing level of PAFT frequency. From the ANOVA statistical test with a real level ($\alpha = 0.05$), it was concluded that the treatment with a certain frequency level had a significant effect, while the exposure time had an insignificant effect on the area of red-lettuce. The result of DMRT with a 5% significance level, it was found that the treatment with the most significant frequency level, was conducted at 3-5 kHz frequency. From these findings, it was apparent that PAFT treatment significantly affected the increase in the area of red-lettuce leaves with the best treatment at a frequency of 3-5 kHz and 2 hours exposure time. Leaf area became one of the morphological parameters to find out the growth in red-lettuce [45]. Hou [46] also proved an increase in leaf area in cotton plants that were treated with PAFT compared to plants without PAFT. Thus, PAFT exposure treatment had a significant effect on the increase in leaf area compared to plants without PAFT.

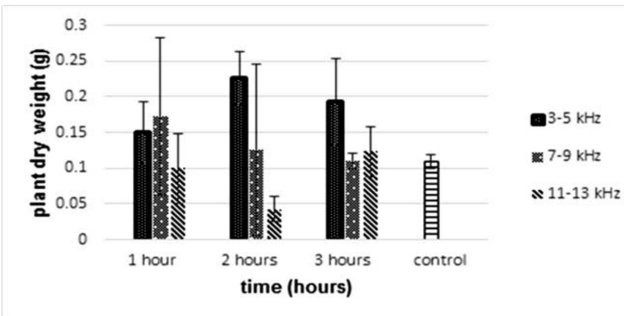


Figure 6. Relationship between variations in frequency and time of PAFT exposure to the dry weight of red-lettuce plants

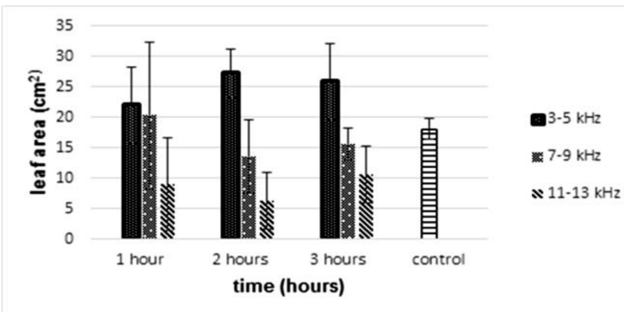


Figure 7. Relationship between variations in frequency and time of PAFT exposure to red-lettuce leaf area

Plant height parameters were also measured to see the effectiveness of PAFT on the vegetative growth of red-lettuce plants. From the obtained results, it was found out that the highest average value was in the treatment frequency of 3-5 kHz and 2 hours exposure time with plant height values of 9.43 cm. In contrast, the lowest plant height parameters were found in the treatment frequency of 11-13 kHz with an exposure time of 2 hours, and a plant height value of 5.16 cm. The graph of plant height observations between each PAFT treatment is shown in Figure 8. The graph indicated that the parameters of PAFT exposure time at 2 and 3 hours had a similar pattern, in which the plant height decreased along with the increasing frequency of PAFT in red-lettuce plants. From the results of ANOVA statistical tests conducted with a real level ($\alpha = 0.05$), it was revealed that only the treatment with the frequency level had a significant effect on plant height of red-lettuce. From the results of DMRT with a 5% significance level, it was found that the treatment with the most significant frequency level was on the frequencies of 3-5 kHz and 7-9 kHz. From these results, it was revealed that the best PAFT treatment was at the frequency level of 3-5 kHz and 7-9 kHz. This result was in line with the study of Singh [47] presenting that PAFT treatment increased peanut plant height with exposure to violin music, where plants with PAFT had better plant height. Exposure to a sound frequency of 0.3-6 kHz for 3 hours daily can increase plant height. These results were also in line with research conducted by Cai [48], pointing out that PAFT at low frequency can significantly increase plant height.

Observations made on the number of leaf parameters indicated that the highest average value was in the treatment frequency of 3-5 kHz and exposure time of 2 hours and 3 hours with an average number of 10 leaves. The lowest number of leaves was at the treatment frequency of 11-13 kHz and the exposure time of 2 hours with a total of 8 leaves. Plants without PAFT treatment indicated the lowest number of leaves compared to plants that were exposed to PAFT. The graph of observations indicating the number of leaves between each PAFT treatment is depicted in Figure 9. The pattern in each treatment during PAFT exposure had a nearly uniform pattern; increasing frequency of PAFT affects the decreasing number of red-lettuce plant leaves. Meanwhile, an interesting pattern was also depicted in the 3-5 kHz PAFT frequency parameter where the tendency for the leaf number tends to increase along with the longer exposure time of PAFT. From the ANOVA statistical test with a real level ($\alpha = 0.05$), it was found that the frequency level had a significant effect on the number of leaves of red-lettuce. From the results of DMRT with a 5% significance level, it was also revealed that the frequency level of 3-4 kHz significantly affected lettuce plants. From these results, it was apparent that the provision of PAFT at the frequency level of 3-4 kHz and exposure time of 2 hours and 3 hours had a significant effect on the number of plant leaves amounting to 10 strands.

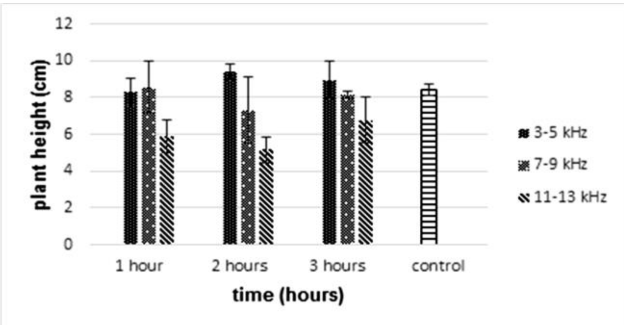


Figure 8. Relationship between variations in frequency and time of PAFT exposure to red-lettuce height

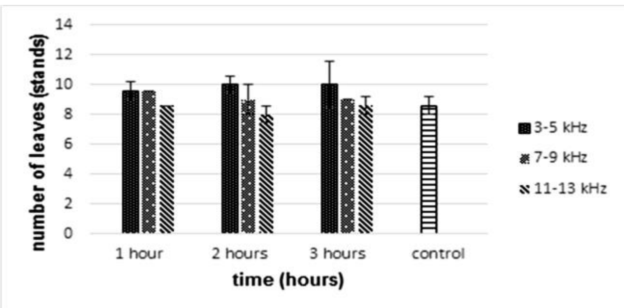
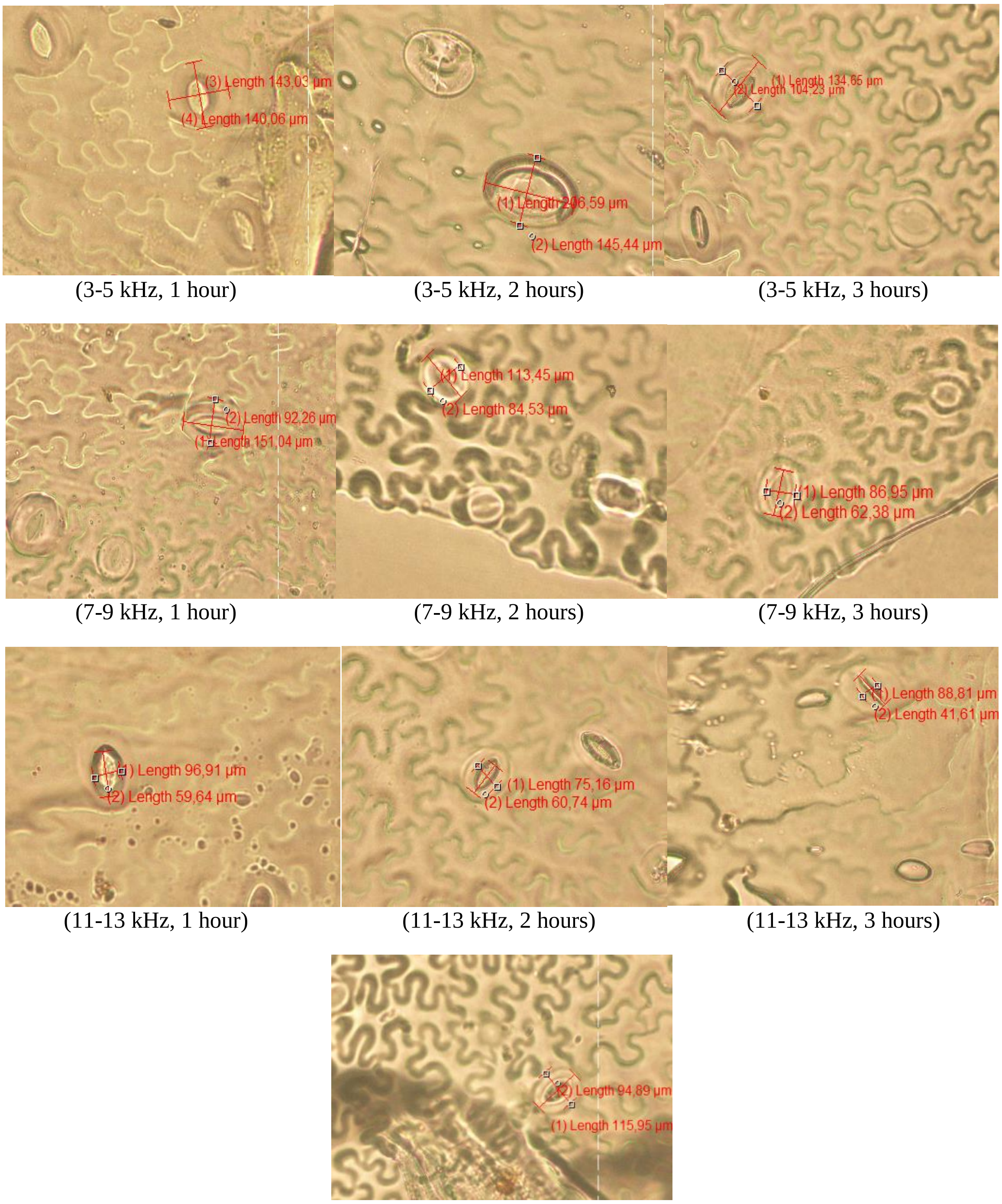


Figure 9. Relationship between variations in frequency and time of PAFT exposure to the number of red-lettuce leaves

Stomata become a plant organ that acts as a pore on the leaf surface to regulate the absorption of carbon dioxide (CO2) in the process of photosynthesis and transpiration. According to Santana [49], turgor change in cells determined the pore area of the stomata, where gas diffusion can occur to maintain a constant internal environment in the leaf. Stomata act as a controller related to the availability of CO2 and efficiency in water use in plants. In this study, the results indicated that stomata in plants that received PAFT treatment opened wider when compared to control plants (without PAFT treatment). Stomata opening width measurement was conducted at the lower part of red-lettuce leaves. The time of collection was performed in the morning, precisely at 15 minutes before PAFT treatment was completed. As seen in Figure 10, stomata openings were observed by using an Olympus CX31 microscope with a magnification of 40×.



(3-5 kHz, 1 hour) (3-5 kHz, 2 hours) (3-5 kHz, 3 hours)

(7-9 kHz, 1 hour) (7-9 kHz, 2 hours) (7-9 kHz, 3 hours)

(11-13 kHz, 1 hour) (11-13 kHz, 2 hours) (11-13 kHz, 3 hours)

Control

Figure 10. Lower stomata part of red-lettuce leaves

The observations indicated that treatment at frequencies of 3-5 kHz and 1-hour exposure time resulted in stomata opening widths ranging from 140.06~143.03 µm, while at 2 hours exposure time the stomata opening widths ranging from 145.44~206.59 µm, and for 3 hours exposure time the stomata width ranging from 104.23~134.66 µm. Results at 7-9 kHz frequency and 1 hour exposure time indicated stomata opening width ranging from 92.26~51.04 µm, while for 2 hours exposure time resulted in stomata opening width ranging from 84.53~113.45 µm, and for exposure time 3 hours ranging from 62.38~86.95 µm. Results at a frequency of 11-13 kHz and exposure time of 1 hour resulted in stomata opening width ranging from 59.64~96.91 µm, while for 2 hours exposure time resulted in stomata opening width ranging from 60.74~5.16 µm, and for exposure time of 3 hours resulted in exposure width ranging from 41.61~88.81 µm. The result of the stomata opening width in control plants was 94.89~115.95 µm. It was thus concluded that the largest stomata opening was in the PAFT treatment with 3-5 kHz frequency which had a better increase in stomata opening compared to PAFT treatment at a frequency of 7-9 kHz, 11-13 kHz, and in control treatment (without PAFT). This was in line with research conducted by Qin [50] and Chuanren [51] which proved that plants were more responsive to PAFT at low frequencies. Leaf stomata that can open more optimally will increase the vegetative growth of plants because the absorption of nutrients and other substances in the leaves becomes more effective and optimal. Overall, it was concluded that the use of PAFT applications can increase the growth of biological objects quantitatively and qualitatively [52-55].

4. CONCLUSION

Based on the results of the study, plant acoustic frequency technology (PAFT) can significantly increase the vegetative growth of red-lettuce. It was revealed that the effect of PAFT was best found in the treatment with a frequency level of 3-5 kHz when compared with the treatment at the frequency level of 7-9 kHz, 11-13 kHz, and in control treatment (plants without PAFT). The best value for the parameter red mean leaves with the most concentrated redness level was in the treatment of 3-5 kHz with a time of 2 hours that was equal to 63. The highest wet weight of the plant was in the treatment of 3-5 kHz and the exposure time for 2 hours with a wet weight of 4 g. The dry weight of plants with the highest average value was at 3-5 kHz frequency and 2 hours exposure time with a dry weight of 0.22 g. The highest leaf area parameters were in the treatment frequency of 3-5 kHz and exposure time of 2 hours with leaf area values of 27.19 cm^2. Plant height with the highest average value was in the treatment frequency of 3-5 kHz and exposure time of 2 hours with a plant height value of 9.43 cm. The highest number of leaves was found in the treatment frequency of 3-5 kHz and exposure time of 2 hours and 3 hours with an average number of 10 leaves. Observation of leaf stomata opening indicated that treatment at a frequency of 3-5 kHz and 2 hours exposure time produced the best width of stomata opening ranging from 145.44~206.59 µm.

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