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THE INCREASE OF STOMATA OPENING AREA IN CORN PLANT STIMULATED BY DUNDUBIA MANIFERA INSECT SOUND

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Abstract:

This study aims i) to determine the effect of Dundubia manifera insect sound on the stomata opening area of corn plant (Zea Mays L.) at frequencies of (in Hz) 3000, 3500, 4000, 4500, and 5000, and ii) to know the peak frequency that can optimize the stomata opening of the corn plant. The insect sound has been manipulated into peak frequencies and validated using Octave 4.2.1 software. The experiment uses one corn-field for the treatment and control plants. Sampling is taken three times, i.e.: 15 minutes before sound exposure, during sound exposure for 30 minutes, and 15 minutes after sound exposure. The stomata opening area is observed using a microscope by observing the output via NIS Elements Viewer program. The length and width of the stomata openings are measured using Image Raster 3.0 and the area of the stomata opening is calculated using the elliptic equation. This study shows that the stomata opening area when given sound exposure is larger than without sound exposure. The largest stomata opening area is obtained at a frequency of 3000 Hz, viz.: 93.7 μm².

Keywords: Dundubia Manifera Insect Sound; Stomata Opening Area; Elliptic Equation; Corn Plants.

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1. Introduction

Stomata are an important part of plants. These are pores formed by a pair of guard cells and control the gas flow and regulates water and CO_2 influxes between the plant and its environment, which is essential for the photosynthesis process [1-4]. Stomata open because the guard cells take up water and swell, which forces the inner cell wall to be pulled [5]. This is due to an increase in the concentration of ions or other solutes within the guard cells because of i) the dynamics of K^+ and Cl^- ions, ii) the metabolism of stored sucrose, and iii) a combination of the two aforementioned processes. Furthermore, the increase of turgor pressure causes the water to enter the guard cells through osmosis due to the increase of the aforementioned concentrations, and vice versa for the

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closing of stomata [6]. Hence, the stomata are important in the growth of the plant. The larger the opening of the stomata the better the flow of fluids between the plant and its environment, making it better for the plant to grow. Furthermore, optimizing the opening of the stomata may increase the growth and eventually the harvest products of the plant.

Dundubia manifera insect [see Fig. 1] is one of the typical insects in Indonesia. The insect usually lives in tropical climates. *Dundubia manifera* has a loud and distinctive sound by swiping both wings and can be found every morning on trees and around rice fields. Now, it is believed by local farmers that the insect sound may as well contribute to the growth of rice plants. Partly based on this believe, we would like to know whether the sound of *Dundubia manifera* affects some parts of plants, especially in this case the stomata of the plants. The original sound of *Dundubia manifera* reaches a frequency of 3300 Hz, which is clearly audible (audio sonic), i.e. may be heard by humans. However, in this case we use a range of frequencies from 3000 Hz to 5000 Hz. Hence, we may not directly use the original sound of the insect. The sound has to be analyzed and manipulated to produce a range of frequencies needed. Hence, the original sound of the insect is recorded using a mic condenser and then analyzed using Octave 4.2.1 Software to give the needed frequencies.

Figure 1: An image of a Dundubia manifera insect [7].

The effect of sound towards plants is an interesting and emerging study at the moment. This is demonstrated by the many studies that have been conducted to investigate the effect of various sound sources towards: i) the metabolism of roots and the synthesis of nucleic acid and protein in *Chrysanthemum* [8,9], ii) the antioxidant enzyme activities and lipid peroxidation of *Dendrobium candidum* [10], iii) the stimulation on the secondary structure of plasma membrane protein of tobacco cells [11], and iv) the energy metabolism of *Actinidia chinensis callus* [12]. Hence, various sound effects are attributed to different parts of the plants. These effects may positively or negatively contributes to the growth and productivity of the plants. Based on the above description, there has not been any study that investigates the effect of insect sound towards the stomata opening of plants. Hence, this study reports for the first time the stomata opening stimulated by the sound of *Dundubia manifera* insect with a variation of the insect's sound frequency. The stomata opening is determined by the area of the stomata based on elliptical geometry calculations. This is conducted because the shape of the stomata opening area is similar to that of the elliptic geometry. To the knowledge of the authors, this study has not been conducted before. Hence, the aims of this study is to i) determine the effect of *Dundubia manifera* insect sound towards the stomata opening area of corn plant, and ii) to determine the peak frequency that may optimize the stomata of corn plant.

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2. Materials and Methods

This is an experimental-type research. The experiment is conducted to determine whether the sound of *Dundubia manifera* insect may affect the stomata opening area of corn plant and also to determine the sound frequency that gives the optimum stomata opening area. The general procedure in this study are i) recording the insect sound, ii) analyzing and manipulating the insect sound to give the needed frequency range, iii) exposing the insect sound to the corn plants, and finally iv) measuring the stomata opening area before, during, and after sound exposure. The tools and materials used in this study are i) the *Dundubia manifera* insect sound source [the black box in Fig. 2], ii) a light microscope [Nikon brand], iii) a laptop with NIS Elements Viewer software, iv) a laptop with Image Raster 3.0, v) glass slides, vi) an alteco glue, and vii) corn plants. Finally, the research site is at a cornfield in Sardonoharjo Village, Yogyakarta Special Region (Indonesia).

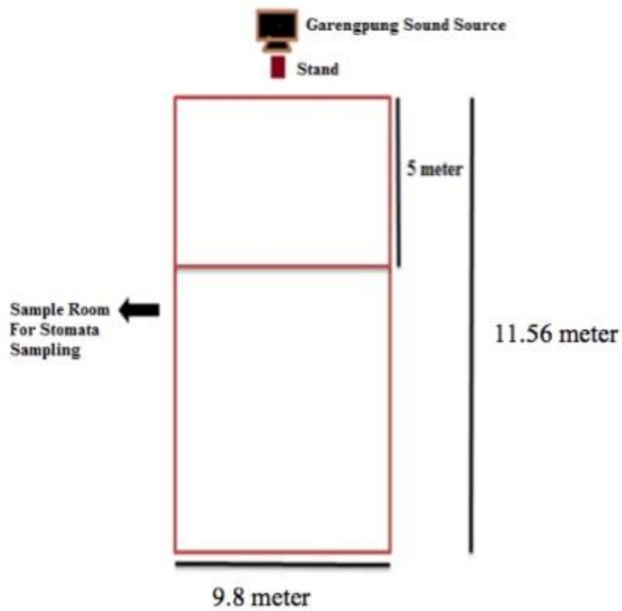

Figure 2: The research design in this study.

The variables in this study consists of i) the insect frequencies of 3000 Hz, 3500 Hz, 4000 Hz, and 5000 Hz as the independent variable; ii) the stomata opening area as the dependent variable, and iii) sound exposure time and distance of the corn plants to the sound source as the control variables. Based on these variables, the research design may be observed in Fig. 2 where the sound source is placed in front of the corn plants with a distance of 5 m between them. The sound source is then exposes sound to the corn plants. The area of the experiment is approximately 11.56 x 9.8 m^2, whereas the area of the cornfield is 6.56 x 9.8 m^2.

Sound exposure of the corn plants is conducted as follows (see Fig. 3-left): i) adjusting the position and distance of the sound source as shown in Fig. 2, ii) adjusting the frequency to be exposed to corn plants to 3000 Hz, iii) turning ON the sound source for 30 minutes, and finally iv) turning OFF the sound source (after 30 minutes). The above procedure from i) to iv) is conducted for other frequencies of 3500 Hz, 4000 Hz, 4500 Hz, and 5000 Hz. The exposure of the insect sound is conducted once for each frequency in the morning from 07.00 to 07.30 o'clock.

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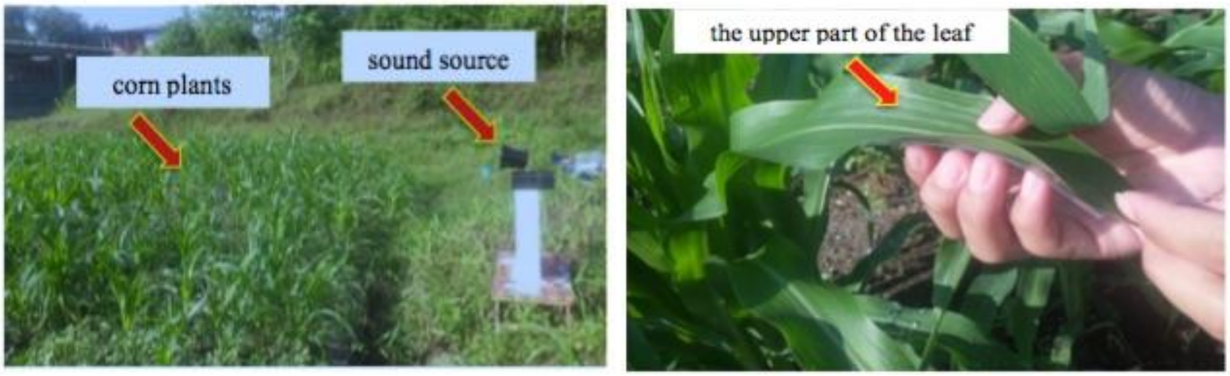

Figure 3: The position of the sound source with respect to the corn plants (left) and the stomata sampling process (right).

Stomata sampling is carried out via the following steps: i) preparing glass slides and the alteco glue, ii) applying the glue thinly on the surface of the glass slides, iii) attaching the glass slides (covered with glue) onto the upper part of the leaf's surface (see Fig. 3-right), iv) gently pealing the glass slides from the leaf, and finally v) the stomata sample is printed on the glass slides (see Fig. 4). Moreover, the stomata samples are taken in three groups, i.e.: i) before sound exposure, (ii) during sound exposure, and (iii) 15 minutes after the sound exposure. As many as 10 stomata samples are taken for each frequency variation in each group.

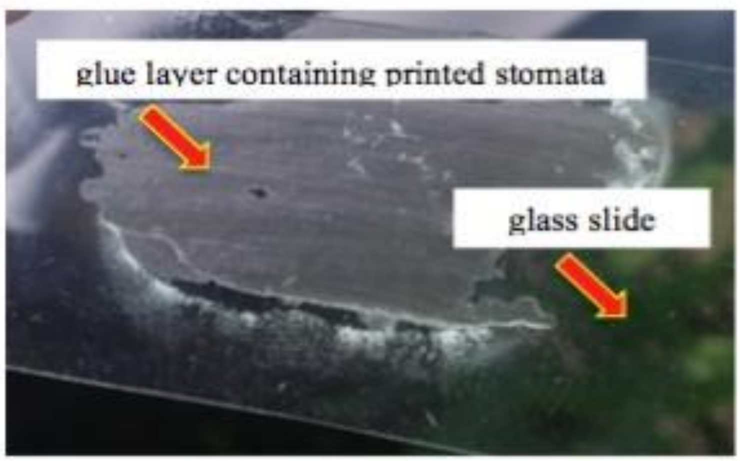

Figure 4: A stomata sample printed on a glass slide covered in glue.

Finally, the last step is to measure the area of the stomata opening. This is conducted with the following steps: i) observing the stomata sample using a light microscope with 1000X magnification, ii) observing the stomata sample via a computer with NIS Element Viewer software until the stomata is clearly visible, iii) saving and storing the stomata images in Jpeg extension, iv) measuring the length [see Fig. 5(a)] and width [see Fig. 5(b)] of the stomata opening via the Raster Image 3.0, and v) calculating the stomata opening area using the elliptical geometry equation, i.e.:

$$A = (\pi/4) \text{ x length x width}, \quad (1)$$

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where A (in μm^2) is the area of the stomata opening and π is a constant with a value of 22/7. Lastly, the stomata opening area samples that have been analyzed using equation (1) are then averaged to obtain the averaged stomata opening area for each frequency and sampling group.

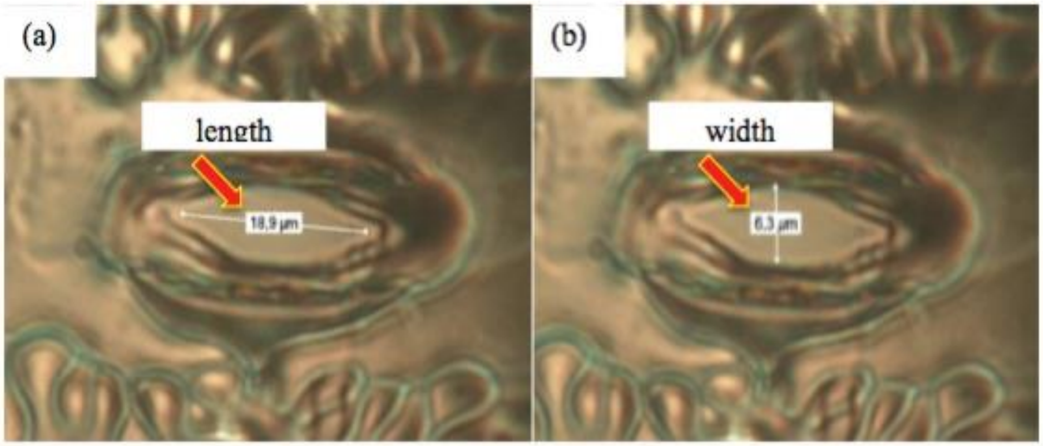

Figure 5: Measurement of the length (a) and width (b) of the stomata opening.

3. Results and Discussions

The original sound wave of *Dundubia manifera* insect manipulated at a peak frequency of 3000 Hz obtained from the sound recording of the insect may be observed in Fig. 6. The sound wave in Fig. 6 is in time-domain. It may be observed that the sound wave is on going as time progresses. Furthermore, the magnitude of the sound wave varies periodically as time progresses. In order to determine the dominant frequency, the sound wave is then analyzed using Octave 4.2.1 Software. The result is the sound wave in frequency-domain given in Fig. 7. It may be clearly observed in Fig. 7 that several peak frequencies occur. However, the dominant or the highest magnitude is obtained at a frequency of 3029 Hz, which is the designated frequency. The same analysis is conducted to obtain the manipulated insect sound at frequencies of 3500 Hz, 4000 Hz, 4500 Hz, and 5000 Hz. These frequencies are then exposed to the corn plants.

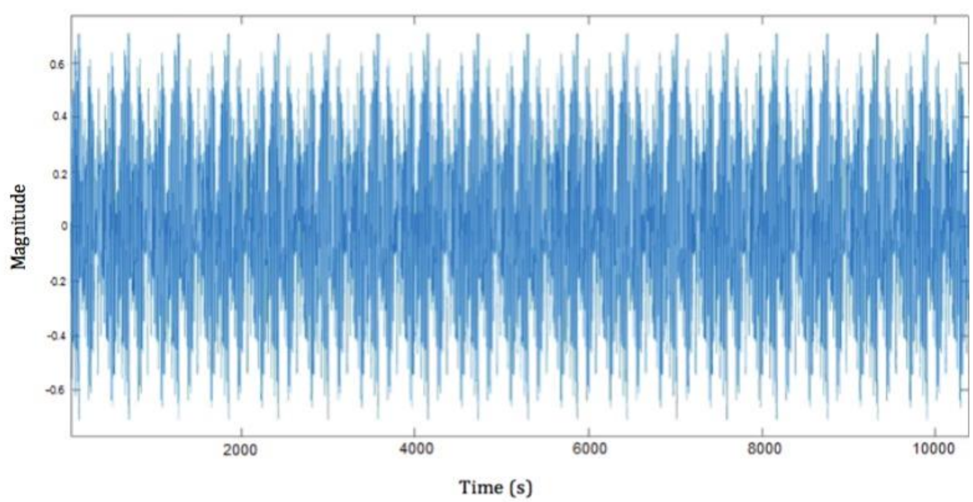

Figure 6: The sound wave of *Dundubia manifera* insect in time-domain.

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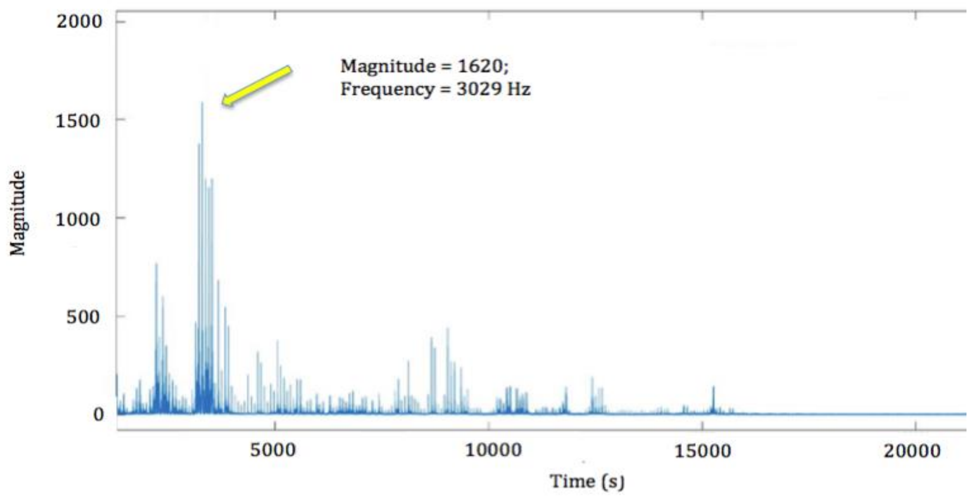

Figure 7: *Dundubia manifera* insect sound at a peak frequency of 3029 Hz.

The stomata that are not exposed to the insect sound can be observed in Fig. 8(a). The stomata are observed as an oval-like tissue where the guard cells are depicted by the top red arrow in Fig. 8(a), whereas the intended stomata opening is showed by a red arrow in Fig. 8(b). The images of the stomata obtained in this study are consistent in structure with that obtained by Voss, *et. al* (2018) where the stomata of *Polypodium vulgare* and *Asplenium scolopendrium* are studied. In this case, all stomata openings are influenced by the mechanism of turgor pressure, the presence of osmotic pressure, and the dynamics of K^+ and Cl^- ions, and environmental factors such as light [14]. However, based on Fig. 8 it may be perceived that the stomata opening area tends to increase during the sound exposure compared to that before the sound exposure indicating that the insect sound does affect the stomata opening of corn plants.

The stomata opening affected by exposure of sound with frequency variations can be observed in Fig. 9. It can be deducted from Fig. 9 that for all frequency variations, larger stomata openings are obtained during the sound exposure (see third column in Fig. 9). Moreover, it can be seen as well that after sound exposure the stomata tend to close again. This qualitatively indicates that sound exposure affects the dynamics of stomata opening.

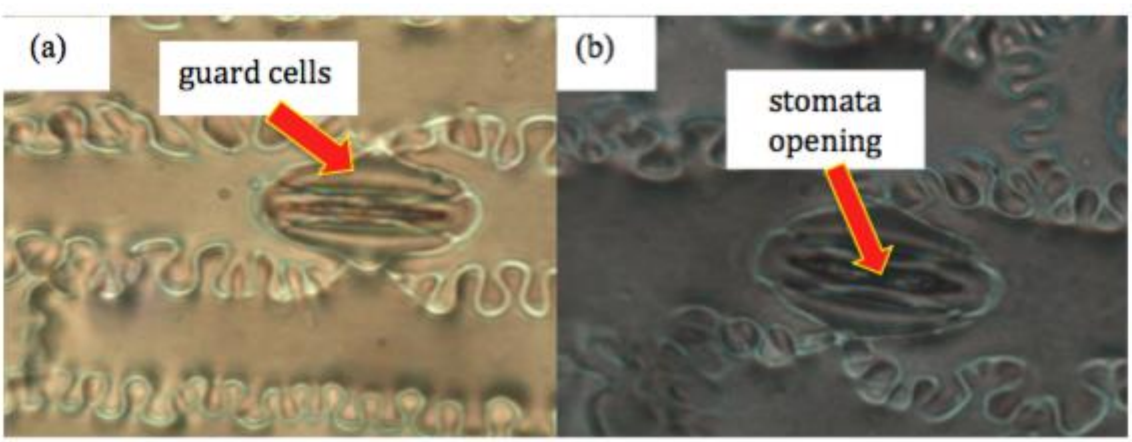

Figure 8: Stomata openings in corn plants before (a) and during (b) sound exposure.

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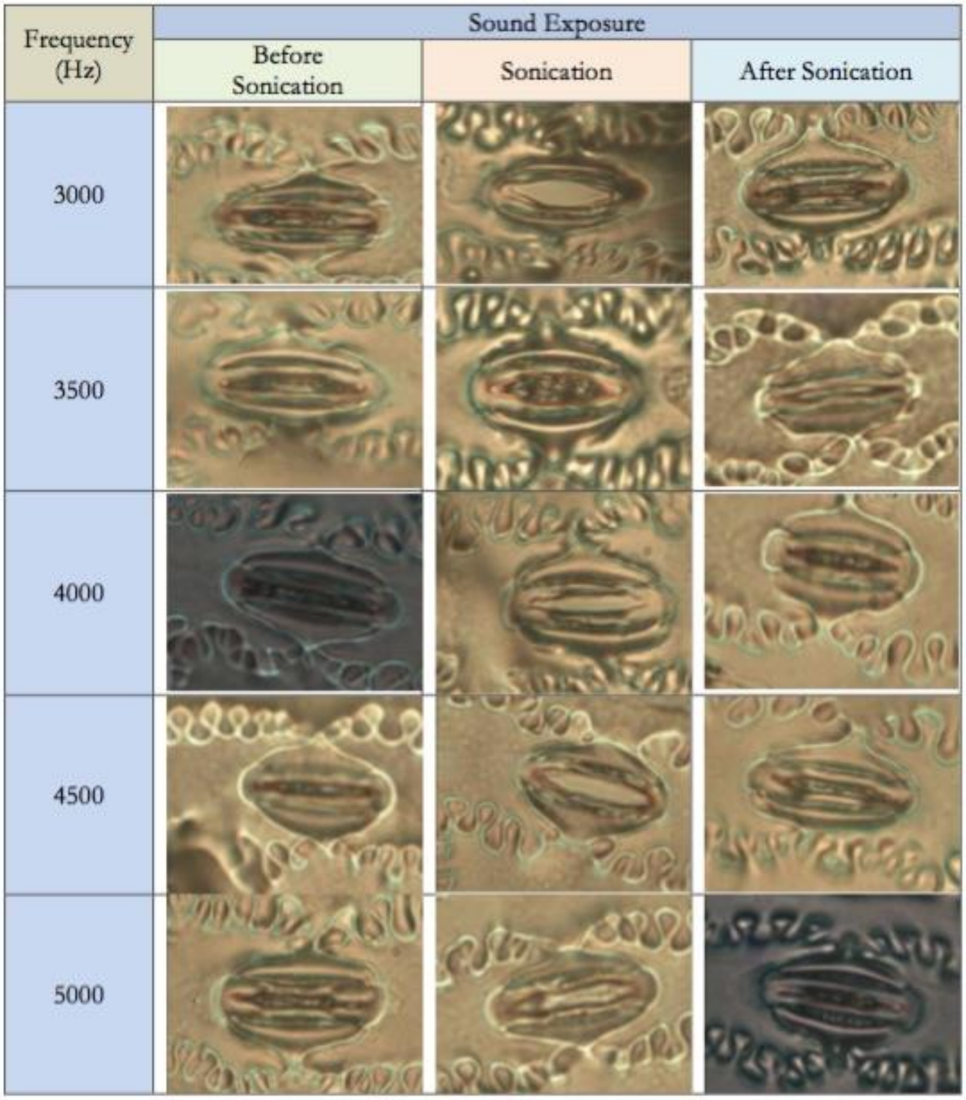

Figure 9: Stomata openings of corn plant leaf samples before, during, and after sound exposure for each frequency variation.

Fig. 10 shows the graph of the stomata area result. The area of stomata openings before, during, and after exposure to sound are indicated by square, circle, and triangle data points, respectively. It can be seen in Fig. 10 that the stomata opening areas during sound exposure are higher than the stomata opening areas before and after sound exposure for all frequency variations. Especially during sound exposure (red line with circle data points), higher frequency (from 3000 Hz to 5000 Hz) tends to make the stomata opening area to decrease, except at 4500 Hz where the stomata opening is increased but still below 3000 Hz. Therefore, 3000 Hz is the optimum frequency to be used in increasing the stomata opening area compared to other frequencies with stomata opening area of 93.7 μm^2 via the elliptical equations. The stomata opening areas obtained from sound exposure of other frequencies are 46.2 μm^2, 37.6 μm^2, 57.9 μm^2, 46.3 μm^2, and 30.7 μm^2 for 3500 Hz, 4000 Hz, 4500 Hz, 5000 Hz, and before sound exposure, respectively. Therefore, sound exposure towards the corn plants with all frequency variations produces larger stomata openings compared to the stomata openings before sound exposure. Especially for frequency of 3000 Hz

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there is an increase of around 3 times of stomata opening area compared to the stomata opening area before sound exposure. Moreover, the stomata opening area after the sound exposure decreases, but still higher than that before sound exposure. This indicates that the insect sound may still give an affect even after the sound is exposed to the corn plants. The stomata opening area obtained in this study is comparable to the stomata size acquired by Dittberner, *et. al* (2018) that is around 90 μm² to 130 μm². However, our results of the stomata opening area is smaller than that obtained by Dittberner, *et. al* (2018). This may be caused by the approximations of the stomata opening area using elliptical calculations.

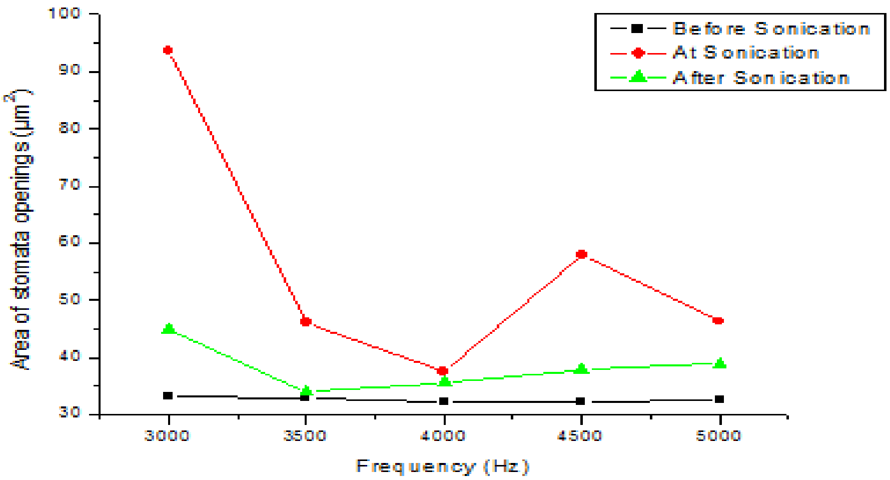

Figure 8: A relationship between areas of stomata opening (μm²) vs sound frequency (Hz).

Here, we propose that resonance is the cause of the stomata opening area increase when the insect sound is exposed to the corn plants. The stomata opening is caused by the sound source that resonates with the liquid in the stomata or the layer of the stomata, which then pushes the guard cell walls to increase turgor pressure so that the stomata opens wider. In this case the optimum resonance for the corn plants is obtained at 3000 Hz. As mentioned above, the insect sound with a frequency of 3000 Hz is the original sound frequency of *Dundubia manifera*. This may indicate that the stomata opening of the corn plants are mainly sensitive to the original insect sound compared to the manipulated ones.

Many studies have used other frequencies to study the effect of sound towards some parts of plants. Yi, *et. al* (2003) used a frequency of 1000 Hz capable of increasing root activity and plasmalemma H^+-ATPase in *Chrysanthemum* plants [16]. Bochu, *et. al* (2003) showed that sound effects with a frequency of 400 Hz is able to increase germination index, stem height, relative increase in fresh weight, rooting ability, root system activity, and ability to penetrate cell membranes in rice seeds [17]. Kim, *et. al* (2015) showed that giving a 1000 Hz frequency sound wave can delay the ripening of tomatoes [18]. The frequencies that have been used in the aforementioned studies are well below 3000 Hz and can have an effect on *Chrysanthemum*, tomato, and rice seed plants. Hence, the stomata opening area increase stimulated by the *Dundubia manifera* insect sound, which ranges from 3000 Hz to 5000 Hz, widens the range of sound frequency that have an effect on plants. There have been no previous studies that use these frequency ranges to be exposed to plants, so that these frequency ranges are used for the first time for sound exposure to corn plants.

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4. Conclusions and Recommendations

Insect sound exposure with frequency variations of 3000 Hz, 3500 Hz, 4000 Hz, 4500 Hz, and 5000 Hz can stimulate the stomata opening area of corn plants than that without sound exposure. The widest stomata opening area is obtained at 3000 Hz, that is 93.7 μm^2, compared to other frequencies. This shows that the frequency of 3000 Hz is the optimum frequency for insect sound exposure to corn plants. Further study in this line of research should include the physical mechanisms of how insect sound affects the stomata opening, which has not been done in this study.

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