Increasing Mustard (*Brassica juncea* L.) Yields through Exposure Sound and Preventive Pest Management Based on Refugia Plants

Asritanarni Munar, Widihastuty, Rini Susanti, Muhammad Hanafi, Imam Hartono Bangun*

Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Indonesia *Corresponding author email: <u>imamhartono@umsu.ac.id</u>

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Abstract. In Indonesia, mustard plants will increase by 8.2% in 2021, but more than that is needed to meet the community's needs due to a growing population. Pests and fertilization issues hinder the anticipated growth, but the application of sound can serve as an alternative to traditional plant fertilizers, as exposing plants to sound can enhance their growth by opening stomata, facilitating water absorption, and promoting increased nutrient uptake. This study aimed to examine the application of sound effects and refugia plants to increase the growth and yield of mustard greens (*Brassica juncea* L.). The study used a Time's Series Randomized Block Design with three replications, and significant data were analyzed using a different test at the 5% confidence level. The first factor is sound applications (S) consisting of S₀ = without sound, S₁ = rock music (Frequency 21-14,000 Hz), and S₂ = classical sound (Frequency 21-14,000 Hz). The second factor is the refugia plant (R) consisting of R₀ = without refugia, R₁ = *Tagetes erecta* L., R₂ = *Zinnia elegans* L, and R₃ = *Ocimum bassilicum* L. The application of classical sound frequency (20-14,000 Hz) significantly affects the growth process of mustard plants, including plant height, leaf count, leaf area, and total chlorophyll. Refugia plants affect the process of plant protection through their ability to attract pests in the research area.

Keywords: music; organic; refugia; yield mustard

INTRODUCTION

The covid-19 pandemic in Indonesia in 2020 significantly impacted the health and socio-economic conditions of the entire community (Suryana et al., 2021). Currently, the need for mustard greens is increasing along with the health crisis that occurred. This health crisis has also caused people to prefer consuming vegetables to increase their immune systems (Sarkar et al., 2022). Indonesians generally like mustard plants as daily food (Amri, 2022). In 2021 the production of mustard greens in Indonesia had increased by 8.2% compared to 2020 (Kadarmanto et al., 2021). However, more than this is needed to meet the community's needs. An important factor causing the decline in production is the attack of plant pest organisms and fertilization problems. Farmers still use large quantities of agrochemicals for plant nutrition and pest control (Purba et al., 2020). Excessive use of chemical fertilizers can harm the soil and the environment (Minasny et al., 2017). In addition to harming living things and the environment, excessive use of synthetic pesticides can cause plant pests to become resistant, build-up chemical residues in crops,

and kill natural enemies (Tatuhey et al., 2020).

The act of fertilizing plants can be replaced by sound treatment (Galina et al., 2022). Applying sound to plants is an activity to improve plant growth using highfrequency (Kadarisman et al., 2019). Besides, it can spur the opening of leaf stomata to become more expansive and make it easier for plants to absorb more water and CO2 in optimizing photosynthesis (Arlius et al., 2021). Sound can control pest attacks (Hou et al. 2010). Insects communicate by emitting sound waves. This can be the basis for destroying insect communication, disrupting the mating process, and reducing the population of plant-disturbing organisms (Nawawi et al., 2020). In addition, refugia planting technology as a trap crop can reduce the intensity of pest attacks. Refugia plants act as a source of food and temporary shelter that can meet the needs of natural enemies because they provide a source of nutrition and energy, such as nectar, powdered honey, and honeydew (Anggraini et al., 2020).

The technology of sound treatment can also increase strawberry production in greenhouses by 6% and reduce pest and disease attacks by 66% (Qi *et al.*, 2009). Increases rice in pots (17.4 - 39.7%), rice in fields (5.7%), wheat (17%), and cucumbers (60%) and can reduce pest attacks and spider mite diseases (6%), aphids (8%), late grey mold (9%), late blight (11%) and viruses (8%) (Hou *et al.*, 2010). Inorganic pesticides should be stopped, and environmentally friendly pest management should be used.

Refugia plants and pest trap pestrepellent plants and attract natural enemies to live and reproduce in the area so that the presence of natural enemies can balance the pest population at a limit that is not detrimental (Asmoro and Winasa, 2021). This research is necessary because, based on previous research, sound can stimulate the encroachment of phosphorus nutrients and soil microbial populations (Munar *et al.*, 2022) and is associated with decreasing pests (Qi *et al.*, 2009). This study aimed to examine the application of sound effects and refugia plants to increase the growth and yield of mustard greens (Brassica juncea L.).

METHODS

Field research was carried out in the Universitas Muhammadiyah Sumatera Utara (UMSU) research field, Sampali Village, Percut Sei Tuan District, Deli Serdang Regency, no sound; N: 3°.666299, S: N: 3°.663550, 98°.711611, classic; S: 98°.710746, rock: N: 3°.663509, S: 98°.713720, elevation; 27 meters (Figure 1) from February to April 2021. From February to April, climatic conditions in the research field (Table 1).

The materials used were mustard seeds of the shinta F1, bougain villaea (Z. *elegans*), marigold plants (T. *erecta*), basil (O. *Bassilicum*), compost (100 kg), bayfolan (250 ml). The tools used in the study included a speaker (TOA ZH-615RM), MP3 player (OEM FEI2915MSD), ruler (Butterfly 30 cm), sound level meter (Fluke 945), scales (analog Q2), thermometer, binocular microscope 40 and 100 (XSZ-107BN 220 V 50-60Hz Karya Graha Agung), oven (Memmert type UN 55), and analytical balance (Henherr type BL-H2 A2000 gr).

Implementation

A factorial times series randomized block design used two factors: The first factor is sound (S) factor has three treatment location points on a land area of 25 ha (Figure 1) with a frequency of 20-14,000 Hz, namely: S₀: Control (no sound), S₁: Music rock ("Avenged Sevenfold-Bad Country"), S₂: Music classic ("Mozart-Brain Power") and the second factor is three types of refugia (R), R₀: Control (without refugia), R₁: *T. tagetes erecta*, R₂: Zinnia elegans and R₃: Ocimum bassilicum. An experimental plot of 100 m⁻¹ consisting of three replications with 36 plots.

The land is cleared of weeds, the soil is loosened, and the mustard seeds are sown in small trays. Then make a plot size of 80 cm x 140 cm, and the area of research land in one location used has a length of 4.4 m with a width of 6.2 m. Then 3 kg of topsoil polybag⁻¹ planted mustard greens and refugia, mixed with compost as much as 108 g polybag⁻¹ in each polybag. Refugia plants were carried out in polybags (20 x 25 cm) by planting seeds of paper flower plants (Z. elegans) and marigold plants (T. erecta) of the same age. Naturally, mustard plants occupy refugia plants. Loudspeakers (TOA) are installed at each corner of the research area at 50 cm from the TOA to the plants.

Sounds are heard on the plants from when they are 7 days old after transplanting until 32 days after transplanting every morning at 07.00 - 09.00 WIB and in the afternoon at 16.00 - 18.00 WIB. In this study, 10-50% germination that grew in each treatment demonstration plot 7 days after transplanting was carried out. Foliar fertilization was carried out 14 days after transplanting with a concentration of 2 ml per 1 liter of water. The attacking pests belong to the order Lepidoptera with the families Noctuidae, Erebidae, Crambidae, and Agromyzidae, which belong to mustard plants' leaf and stem borer larvae.

Data analysis

The research data were analyzed twice, and the first used a non-factorial randomized block design to see the ability of refugia (2). The second analysis combines the three sound locations to compare treatments between sounds (3). According to Duncan's multiple range test, significant data were analyzed using a different test at the 5% confidence level (Weber and Skillings, 2018) e-ISSN 2655-853X https://doi.org/10.37637/ab.v6i2.1219

$$Y_{ij} = \pi + \beta_i + R_j + \epsilon_{ij} \tag{2}$$

- Y_{ij} : Observation values refugia treatment at j-level and repetition at i-level
- π : General average of observed values
- R_j : The effect of refugia treatment at jlevel
- β_i : The effect of repetition at i-level
- ϵ_{ij} : The effect of error refugia treatment and replication

Period	Precipitation (mm day ⁻¹)	Rainy Day	Max. Temp (°C)	Min. Temp (°C)	Av. Temp (°C)	Humidity (%)	Sunlight (%)
Februari	22	2	32.1	24.3	27.4	82%	69%
Maret	38	10	32.3	22.1	26.4	87%	12%
April	73	15	32.8	24.6	27.6	83%	16%

Table 1. The annual mean precipitation, rainy days, temperature, humidity and sunlight

Source: (Klimatologi, 2021)

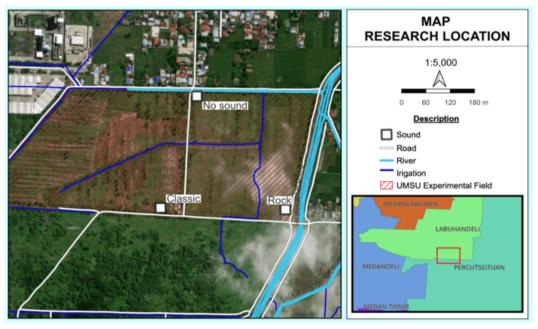


Figure 1. Research location map

The linear model for combination analysis:

$$Y_{ijk} = \pi + S_i + \alpha_j + \beta_k + (S\beta)_{ik} \qquad (3) + \epsilon_{ijk}$$

Information

- Y_{ijk} : Observation sound at i-level, refugia treatment at j-level and repetition at k-level
- π : General average of observed values
- S_i : Effect of location treatment sound treatment at i-level
- α_j : The effect of repetition at j-level
- β_k : The effect of refugia at j-level on the sound location treatment at i-level
- $(S\beta)_{ik}$: The interaction effect of sound at level and refugia at k-level
 - \in_{ijk} : The effect of error sound treatment, replication and refugia.

RESULTS AND DISCUSSION

Plant height

Here we investigate the magnificent effect of sound application and refugia plans as preventive pest management for green mustard's emergence, survival, and growth responses. In each trial, either sound or refugia were run alongside each treatment for combinations. Observations were made once every three days from 7 to 25 DAT and showed that the music application (S₂) affected the exaltation mustard. The results analyzed alongside ANOVA show that sound treatment in the form of rock music (S₁) or classical music (S₂) can stimulate the growth of mustard plants faster (Figure 2).

The influence of musical factors, such as frequency, wavelength, and amplitude, can affect the attraction or repulsion of pests in attacking plants. In research conducted, it is known that certain frequencies have been able to repel flying pests, such as 1.2 KHz repelling four plant hopper of a particular pest, 1.5 KHz repelling five plant hopper, and 27 KHz repelling one plant hopper. Moreover, specific wavelengths in music can also impact the response of pests to plants, where some pests are more sensitive to particular wavelengths, resulting in varying responses to the music. Additionally, high amplitude in music can alter the perception of pests about their environment and consequently affect their behavior towards plants. The environmental conditions during rain, the responses during day and night, and the differences based on the insects' sexes have not been observed yet.

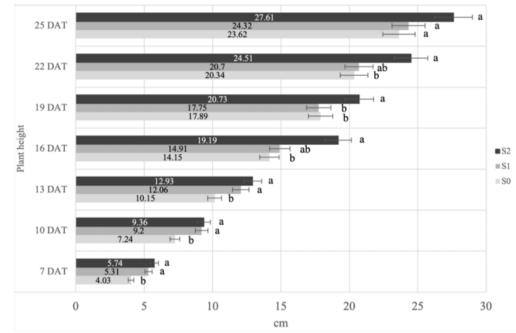
Different sound applications influence the growth of mustard plants, and classical music sound has the best impact at each observation time. Starting from 16 DAT, the height growth of mustard plants with the application of classical music (S_2) is much higher up to 25 DAT compared to no sound (S_0) and rock music (S_1). Classical music (S_2) increases by 17.01% higher than without sound application (S_0) and 15.6% higher than rock music (S_1) application. Based on (Hassanien *et al.*, 2014), sound stimulation can accelerate the combination of Ca²⁺ and vacuole membranes, and alternative voltages can affect the opening and closing of Ca²⁺ channels, which causes changes in ion concentrations on both sides of the membrane and membrane potential, causing plant tissue growth. The mechanism of sound waves promotes plant growth, and there are three possible reasons: environmental stress (including sound wave stimulation) alters the fluidity and permeability of the membrane, Ca²⁺ signalling molecules generate stress signalling to other signalling molecules, propagation of stress signals cause expression of associated genes (Yiyao et al., 2000).

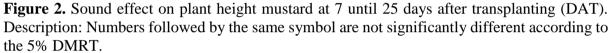
Number of leaves

The test results on the mustard plant's number of leaves with the application of sound and refugia plants significantly impacted the number of leaves at the age of 7 and 22 DAT. This shows that the application of sound and refugia plants stimulates the increase in plant leaves but does not form a significant interaction between the two treatments.

The sound increases the number of leaves of the mustard plant significantly, and the use of classical music (S_2) is the best sound treatment. Classical music can increase the number of plant leaves because the frequency of classical music can stimulate the production of various mustard plant growth hormones.

Several researchers argue that acoustic sound frequency can significantly stimulate the production of endogenous hormones, such as IAA, GA, and ZR (Zhu et al. 2011), also increase the production of and vegetables, including tomatoes (Meng et al., 2012), muskmelon, cowpea and eggplant (Jiang and Huang, 2012). Meanwhile, the applications create rock music chaos preventing the plants from fully growing, sound waves vibrating plant leaves, and accelerating the movement of protoplasm within cells (Chivukula and Ramaswamy, 2014).





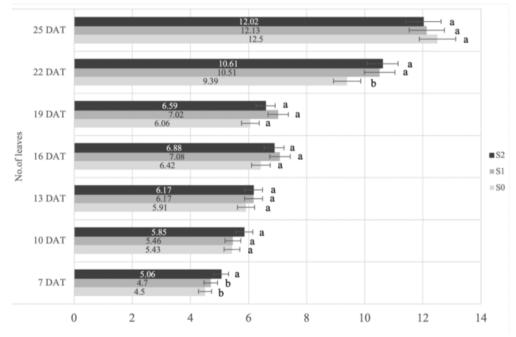


Figure 3. Sound effect on the number of leaves at 7 until 25 days after transplanting (DAT). Description: Numbers followed by the same symbol are similar according to the 5% DMRT.

Leaf area

Leaf area measurement is performed using the Geometric method by measuring the length and width of the plant leaves. After sound application and refugia plants, the leaf area showed significant results aged 7 and 22 DAT. Classical music showed the highest results in leaf area compared to rock music without sound application, and sound can stimulate the growth of the leaf area (Figure 4).

Sound waves make significant changes to the structure of membrane proteins. The secondary structure of membrane proteins is susceptible to sound wave stimulation, and changes in the secondary structure of membrane proteins can cause an increase in plasma membrane fluidity (Hassanien *et al.*, 2014). A study by (Tao *et al.*, 2001) found that various sound waves can stimulate the synchronization of tobacco cell division and promote DNA synthesis at the stage of cell division. The membrane's fluidity also increases under sound stimulation of multiple sound strengths and frequencies (Zhao *et al.*, 2002). Thereby enhancing plant growth and development. In another study (T Hou et al. 2009), sound frequencies up to 40 dB for 3 hours every day can improve cucumber, sweet pepper, and tomato yield and reduce disease incidence. Interesting findings are significant and can replace chemical preservatives or any genetic alterations. Sound also stimulates the stomata opening and absorption of pesticides and fertilizers, thereby reducing the application of chemicals (Carlson, 2013).

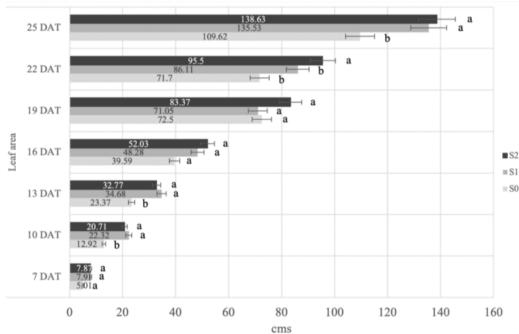


Figure 4. Sound effect on leaf area at 7 until 25 days after transplanting (DAT). Description: Numbers followed by the same symbol are not significantly different according to the 5% DMRT.

Insect identification

Sound effects in planting mustard greens are insects in the mustard planting area and around refugia plants. This can be seen in the sound treatment of rock music (S_1), being able to present as many as 407 insects. In the sound treatment of classical music (S_2), as many as 560 more insects than without sound treatment, which presents as many as 366 insects (Table 2). The identification of insects observed in the planting area resulted in the number of insects with the sound of classical music (S_2) found 560 insects consisting of 450 insects classified as pests, 95 insects classified as predators, and 15 insects classified as parasitoids. Meanwhile, in the sound of rock music (S_1) , there were 407 insects consisting of 302 insects as pests, 90 insects as predators, and 15 as parasitoids. In contrast to planting mustard greens without sound (S_0) , only 365 insects were found, consisting of 277 pests, 79 predators, and 9 parasitoids. Accordance research conducted by (Manullang, 2012) states that exposure to ultrasonic waves can cause changes in eating and progression behavior and stimulate insects to land on plants.

Table 2 shows that the frequency of sound waves generated from rock music and classical music has more insects appearing than without sound. This is to the literature (Prasetyo dan Lazuardi 2019), which states that each insect responds at different frequencies despite coming from the same order. In addition, changes in the frequency of exposure will have a different effect on the behavior of insects.

The types of insects with the status of pests, predators, and parasitoids in the sound treatment of rock music and classical music sound treatment were more diverse than the non-sound treatment, and the number of pests was higher than the number of predators and parasitoids.

The literature (Perkasa, 2020) states that the more heterogeneous a physical environment is, the more complex the flora and fauna community in that place is, and the higher its species diversity. According to research by (Pirngadi *et al.*, 2010) from the results of testing to repel planthopper pests at a frequency of 36 kHz, 40 kHz, 44 kHz, and 8 kHz, this frequency is too high, so it cannot be used to repel planthopper pests in rice plants.

Family	Ordo	Status	S_0	S_1	S_2
Chrysomelidae	Coleoptera	Pest	120	148	195
Cocciellidae	Coleoptera	Predators	18	23	21
Noctuidae	Lepidoptera	Pest	1	2	3
Erebidae	Lepidoptera	Pest	2	1	6
Crambidae	Lepidoptera	Pest	110	102	173
Agromyzidae	Diptera	Pest	30	34	37
Tachinidae	Diptera	Parasitoid	1	2	2
Acrididae	Orthoptera	Pest	14	15	18
Coreidae	Hemiptera	Pest	0	0	3
Aleyrodidae	Hemiptera	Pest	0	0	11
Pentatomidae	Hemiptera	Pest	0	0	2
Pyrrhocoridae	Hemiptera	Pest	0	0	1
Cercopidae	Hemoptera	Pest	0	0	1
Ichneumonidae	Hymenoptera	Parasitoid	8	13	13
Formicidae	Hymenoptera	Predators	23	28	28
Lycosidae	Araneae	Predators	35	36	35
Salticidae	Araneae	Predators	3	3	11
Total			365	407	560

Table 2. Identification of insects on refugia and mustard greens

Description: The results of insect identification based on the sound treatment of different music (S_0 : no sound, S_1 : rock music, and S_2 : classical music).

Pest attack intensity

The observations found that pests attacked the highest sound treatment compared to without sound in combination with all refugia plant treatments. The using sound applications and refugia plants have not significantly affected the intensity of *Phyllotreta vittata* pest attacks (Figure 6). The intensity of pest attacks continues to increase at each plant age, both in the treatment of music sound and refugia plants. In the treatment of refugia plants, the highest pest attack intensity was obtained in the *Z*. *Elegans* treatment (68.48%) and the lowest in the *O. basilicum* treatment (59.38%).

The high intensity of pest attacks on the Z. *Elegans* treatment is because the plant has a lush plant shape, so it is liked by various insects, not only predators, but insect pests also like it as a shelter. Research by (Kurniawati and Martono, 2015) states that refugia are a plant that functions as a microhabitat for natural insect enemies and also help attract plant pests. Research by (Altieri and Nicholls, 2018) shows that the higher the diversity of plants in a community, the higher the abundance and diversity of natural enemies.

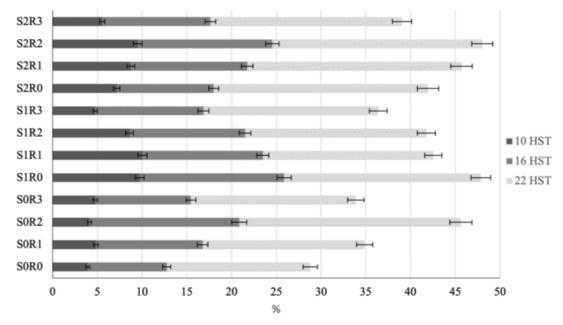


Figure 5. The intensity of *phyllotreta vittata* and the application of sound and refugia plants

Stomata and biomass

Observation of the number of stomata carried out by sound application and the use of refugia plants and their combinations did not show a significant effect. Likewise, the application of sound and refugia plants and their combinations did not significantly affect observing plant biomass. Plant growth development after sound application increased compared to no sound application

Table 3 shows that rock music has the highest number of stomata (S_1) with an average of 699.33 stomata/mm² (10%), much more than classical music. Music can widen the openings of leaf stomata thereby

accelerating the absorption of water, minerals, and CO2 for photosynthesis (Prasetyo and I. B. Lazuardi, 2017). Sound frequency technology stimulates the opening of leaf stomata. Thus, plants can increase the uptake of spray fertilizer and dew (Carlson, 2013). Apart from absorbing water and accumulating photosynthetic products, an adequate supply of nutrients also affects the wet weight of plant shoots and roots. The growth of the eggplant canopy's fresh weight increased with the NPK fertilizer dose at 20-30 DAT (Zulkifli *et al.*, 2020).

The wet weight of the plant canopy is affected by water absorption and the

accumulation of photosynthetic products. The effect of sound application has an impact on the wet weight of the plant canopy but is not significant. The sound of classical music (S₂) shows a higher canopy wet weight compared to no sound (S_0) at 2.52% and rock music (S_1) at 40.02%. The reason is the absorption of more water and CO₂ and optimizing the process of photosynthesis so that plant growth and productivity can be optimally increased (Murni et al., 2018). Sound technology is known to stimulate growth and increase spinach yield by 22.7% and 22.2%, as well as the sugar content of spinach by 37.5%, and vitamins A, C, and B, respectively, increase by 35.6; 41.7 and 40.0%. ;(Jiang and Huang, 2012).

In the greenhouse test, the average weight of the three lettuce species treated by

Agri-wave technology was 44.1% greater than that of the control (Hou and Mooneyham, 1999). The wet-weight roots of the mustard plant by refugia-type treatment and sound application showed a difference in comparison of 1.24% to 4.99%, with the results of the treatment R₀ being higher than R_1 , R_2 , and R_3 (Table 3). Absorption of nutrients in the soil after being given sound applications is thought to increase. Sound waves moving in the soil medium around the roots can stimulate the development of roots to grow bigger. According to research (Bahtiar et al., 2015), applying sound wave technology to rice plants can accelerate the growth of seedlings, extend the roots of rice seedlings, and increase the number of rice seedlings in the nursery process.

Table 3. Total stomata and biomass of mustard plants on 32 DAT

Treatment	Stomata	Header wet	Root wet	Head dry	Root dry
Heatment	(unit/mm ²)	weight (g)	weight (g)	weight (g)	weight (g)
S_0R_0	591.33	70.17	5.56	5.97	0.87
S_0R_1	619.33	54.73	4.44	5.19	0.69
S_0R_2	717.33	32.79	3.66	3.30	0.64
S_0R_3	600.67	85.35	4.90	5.33	0.66
S_1R_0	773.33	43.45	4.24	5.84	0.90
S_1R_1	682.67	38.60	4.26	4.82	0.85
S_1R_2	734.67	46.90	5.01	6.14	1.04
S_1R_3	606.67	31.13	4.61	4.44	0.80
S_2R_0	611.33	53.16	5.27	4.76	0.75
S_2R_1	587.33	60.87	5.81	6.38	0.96
S_2R_2	558.00	71.59	5.64	7.70	0.89
S_2R_3	760.67	63.72	5.06	8.49	0.85

Table 3 shows that after applying sound and refugia plants, the highest dry canopy biomass yield of mustard plants was in the R_2 type *Z. elegans* treatment compared to the R_0 and R_1 treatments of 0.91% to 4.20% for all types of sound. The applied sound has a positive effect on increasing plant biomass. The sound of classical music can increase 27.5% of the highest dry biomass of plants compared to no sound.

Sound stimulation significantly lowers the phase transition temperature and cell growth rate (Zhao *et al.*, 2002), causing the air around the plants to vibrate and can affect the movement of carbon dioxide around the plants affect the absorption of carbon dioxide around the leaves. Table 3 shows the average results of the sound application treatment, and refugia plants showed insignificant results. However, the highest yield of root dry weight was found in the rock music treatment combined with Z. *elegans* (S₁R₂) of 1.04 g.

Mujawwad sound applications with an amplitude of 50 Hz and frequency of 21-17420 Hz can increase corn's available P and K total soil growth in all measurement components (Munar *et al.*, 2023). The plant responds and emits sound in various intensities, frequencies, and duration due to different proses (del Stabile et al. 2022). Sound with a frequency of 20 Hz-20 kHz is reported to affect seed germination, plant growth, cell cycle, and plasma membrane architecture (Ghosh *et al.*, 2016). The evidence shows that sound applications promise to improve agriculture and biotechnology research (Bhandawat and Jayaswall 2022).

Chlorophyll and nutrient content

The sound that has been applied has a significant effect on the total chlorophyll, while the use of refugia plants and their combinations has no significant effect. Observation of the percentage of NPK on the plant after using sound and refugia plants and their combinations had no significant effect (Table 4).

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Sound	Klorofil (CCl)	N (%)	P (%)	K (%)
\mathbf{S}_0	36.66 ab	3.00	0.49	0.53
\mathbf{S}_1	34.61 b	2.08	0.63	0.54
\mathbf{S}_2	41.48 a	4.35	0.41	0.42

Table 4. Total chlorophyll and NPK nutrient content on the plant at 32 DAT

Description: Numbers followed by the same symbol are not significantly different according to the 5% DMRT.

Based on Table 4, the application of sound affects the chlorophyll in mustard plants, with the highest total chlorophyll found in the sound treatment of classical music (S₂), which reaches 9.7% to 13.5% higher than without sound treatment and rock music. The process of opening stomata due to the application of sound stimulates leaf chlorophyll. This is to the research of (Sigmarawan *et al.*, 2020), which states that giving gamelan music to *Gong Kebyar* can stimulate the opening of leaf stomata so that chlorophyll is more optimal in absorbing light, carbon dioxide, and nutrients used for the photosynthesis process.

Then, the rock music application found the highest phosphorus and potassium levels compared to the treatment without sound application. The sound application provides a higher percentage level of the nutrient content of 28.86% compared to that without sound application. In addition, the results of a threeyear experiment on sound technology for rice production showed that acoustic technology could reduce the amount of fertilizer by about 25% (Hou *et al.*, 2010). In accordance with research by (Munar *et al.*, 2020) the application of sound affects the growth process of phosphate-dissolving microbes, as indicated by the discovery of phosphatedissolving fungi in the soil where sound is applied.

CONCLUSION

The application of classical sound frequency (20-14,000 Hz) affects the growth process of mustard plants through the increase in plant height (14.5%), leaf area (20.92%), wet weight (3.5%), dry weight (26.41%), total chlorophyll (11.62%) and plant N content (31.03%). Meanwhile, rock music frequency (20-14,000 Hz) can increase plant height (2.97%), leaf area (19.11%) and total stomata (9.6%). Refugia plants affect the process of plant protection through their ability to attract pests in the research area.

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