# Monitoring growth and physiological responses of *Satureja hortensis* L. to music and noise stimulation

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## Abstract

This research attempted to gain a better view of music/sound interaction with the plant systems. Savory (*Satureja hortensis* L.) seedlings were exposed to the diverse sound treatments, including a piece of Iranian Music (IM), electro-industrial Rock Music (RM), and Urban/Traffic Noise (UTN). The frequencies of IM, RM, and UTN ranged in 800–2000, 1100–7000, and 800–2000 Hz, respectively. The exposure time was three times a day for 45 minutes during two weeks. The applied treatments enhanced shoot dry weight by an average of 55.25% over the control. The IM treatment was the most effective way to improve stem length. The longest root length was observed in RM-treated seedlings (mean 2.3-folds). Root dry weight in IM and RM treatments increased by an average 64.39% and 78%, respectively. Applying music also augmented the concentrations of chlorophyll pigments approximately by 32%. Moreover, carotenoid content displayed a similar increasing trend (mean=36.5%). Likewise, the applied music and sound treatments upregulated the activities of peroxidase (mean=51.33%) and catalase (mean=21.27%) enzymes, implying stimulation in the enzymatic antioxidant system. However, the amount of total sugar was adversely influenced by the treatments (mean 30%). The exposure to UTN was associated with a moderate increase in the proline concentration by a mean of 66% whereas the music treatments exhibited less increase in this parameter. These findings support the view that both music and sound not only affect plant growth programs but also influence diverse biological processes.

**Keywords:** alternating stress, defense system, *Satureja hortensis*, secondary metabolites, sound stimulation

**Azgomi, S., A. Iranbakhsh, A. Majd, M. Ebadi, Z. Oraghi Ardebili. 2021.** 'Monitoring growth and physiological responses of *Satureja hortensis* L. to music and noise stimulation'. *Iranian Journal of Plant Physiology* 11(5), 3911-3918.

## Introduction

Numerous external perturbations are perceived by plants' primary sensory mechanisms that translate the physical and chemical environmental terms such as light, heat, wind, and touch into biological signals (Lamers et al., 2020). Sound waves are another significant extrinsic factors that can elicit plant responses (Kim et al., 2018). Almost all living organisms are immersed in and interact with a variety of sound waves (Chowdhury et al., 2014). Sound is a type of mechanical radiant

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*Received: July, 2021*
*Accepted: October, 2021*

energy transmitted by longitudinal pressure waves in a material environment (Altuntas and Ozkurt, 2019). Music is a harmonious and coherent sound consisting of different frequencies and vibrations. The audible frequency range is between 20 and 20000 Hz (Chowdhury and Gupta, 2015).

Previous studies confirmed that unicellular organisms are also sensitive to music stimulation, and some physiological changes are observed in their behavior (Bochu et al., 2003; Exbrayat and Brun, 2019). Like other living organisms, plants are sensitive to different sounds (Gagliano et al., 2012; Chowdhury and Gupta, 2015). In recent years, numerous mechanic-sensory channels have been identified that are located in the plant plasma membrane. It is assumed that these channels are involved in the perception of a variety of external stimuli (Nejat and Mantri, 2017; Cheung et al., 2020; Lamers et al., 2020).

Existing evidence shows that music may exert distinct effects on plants in terms of physiological performances, growth, and developments, along with increasing levels of polyamines and protein contents, and may lead to their resistance against disease (Joo-Yeol et al., 2015; Choi et al., 2017). However, the optimal sound which can influence plants is varied, depending on several certain factors such as plant species, frequencies, intensities, and duration of the exposure in the application procedures. It seems that plants distinguish a typical sound of an herbivore for the elicitation of defense response (Mishra and Bae, 2019).

Savory (*Satureja hortensis* L.) is an aromatic plant that belongs to the Lamiaceae family, with widespread distribution in the Mediterranean region, West Asia, Europe, Middle East, North Africa, and South America (Çoban et al., 2018). The genus savory has been traditionally used for antiseptic effects, and its therapeutic values have also been reported in the last decade. Savory essential oil includes carvacrol, phenol, thymol, and flavonoids. The existence of a high amount of these components is known as antioxidant property (Momtaz and Abdollahi, 2010; Mahboubi and Kazempour, 2011).

Consistent with the previous studies, this experiment was an attempt to address the influence of music and sound on *S.hortensis* in terms of growth and physiological traits by considering the frequency ranges involved in the treatments (music and noise). The experiment aimed to investigate the responses of savory to different types of music and sound, including Iranian Music (IM), industrial music in Rock genre (RM), and Urban/Traffic Noise (UTN) under the controlled conditions.

## Material and Methods

Seeds of *S. hortensis* were purchased from Pakan Bazr (Isfahan, Iran). Seeds were planted in pots containing a mixture of vermiculite, perlite, coco pit with a ratio of 2:2:1, respectively (EC: 1.2-1.5 $ds.m^{-1}$; pH: 6.5-7). Fourteen-day-old homologues seedlings were selected for exposure to the sound treatments.

The acoustic condition was provided by making four separate growth chambers, with dimensions of 60 × 60 × 90 cm. The pots were located in radial arrangement around the speaker at a distance of 25 cm. Sound treatments were performed for 45 minutes at intervals of three times a day for 14 consecutive days. All pots were incubated under a controlled condition with light intensity 92 µmol photon $m^{-2}s^{-1}$ (16 h light / 8 h dark), and temperature of 25 ℃. Thirty-day-old seedlings were harvested, and various growth-related characteristics as well as the biochemical analysis were measured. All sound treatments were analyzed with sound meter application (Spectrum RTA - audio analyzing tool version 2.10, **Error! Reference source not found.**).

### *Determination of photosynthetic pigments*

The photosynthetic pigments including Chlorophyll *a*, Chlorophyll *b*, Total Chlorophyll, and Carotenoids were determined according to Lichtenthaler (1987).

### *Preparation of enzymes extracts*

Enzyme extracts from leaves were prepared at 4 ℃ using a phosphate buffer (0.1 M, pH 7.5), containing Na2-EDTA 0.5 mM and ascorbic acid 0.5 mM. The homogenates were centrifuged for 15 min at 4 ℃ and supernatants were used as enzyme extracts (Asgari-Targhi et al., 2018).

***Assay of peroxidase (POD) activity***

Peroxidase (EC 1.11.1.X) activity was determined as described by Korio et al. (1989). Enzyme extracts were added to the reaction mixture, including 0.2 mM acetate buffer (pH 4.8), 0.1% (v/v) $H_2O_2$, and 0.04 M benzidine in 50% (v/v) methanol. The reaction was recorded by adding the enzyme extract to the reaction mixture. The peroxidase activity was expressed as an increase in absorbance at 530 nm (Unit $Eg^{-1}$fw).

***Estimation of catalase (CAT) activity***

Catalase (EC 1.11.1.6) activity was determined as the decrease in absorbance at 240 nm (Pereira et al., 2002). Enzyme extracts were added to the reaction mixture, consisting of 50 µM Tris buffer (pH 7.5) and 0.1% (v/v) $H_2O_2$. Differences in absorbance at 240 nm were recorded for three minutes. Finally, the enzyme activity was expressed in Unit $Eg^{-1}$fw.

***Measurement of protein content***

Protein content was measured using Bradford reagent (1976). BSA was used as a standard. Protein content was expressed in mg $g^{-1}$fw.

***Measurement of proline content***

Proline content was measured according to the method presented by Bates et al. (1973). Proline was extracted using sulfosalicylic acid (3% w/v). The mixtures of extract, glacial acetic acid, and ninhydrin reagent were kept in a boiling water bath for an hour. After cooling and vigorously mixing with toluene, this phase was used for measuring absorbance at 520 nm. Proline contents were determined based on the proline standard curve, and finally expressed in µmol $g^{-1}$fw.

***Total soluble sugar assay***

Total soluble sugar was determined based on Watanabe et al. (2000). Leaves from the second node of the stem were selected and homogenized in ethanol 80%. The homogenate was centrifuged for 10 minutes at 6000 rpm, and then the supernatant was used as an extract. The reaction mixture, including freshly prepared Anthrone reagent, and the extract reacted for 10 minutes at 100 ℃. After cooling on ice, the total soluble sugar content was measured at 620 nm. Glucose was used to draw the standard curve. Finally, total sugar content was expressed in mg $g^{-1}$fw.

## Statistical Analysis

Data obtained from this experiment were subjected to analysis of variance (One-way ANOVA) using Graph Pad Prism version 8. The mean values of three independent replications were evaluated by Tukey's test at a significant level ($p<0.05$).

## Results

The application of music and sound treatments promoted seedling growth and performance. Growth indices in 30-day-old seedlings including root length, stem length, and shoot dry weight were influenced by treatments. In comparison with the untreated control, IM and RM significantly improved root length by 83.67% and 122.36%, s, respectively over the control ($p<0.05$) while UTN did not make a significant difference from the control ($p<0.05$) (Fig. I. a). Moreover, stem length was affected by music treatments, and IM led to a significant stem length increase by an average of 38.35%. Exposure to the RM and UTN treatments slightly increased stem length by a mean of 18.05% and 17.15%, respectively while these treatments (RM and UTN) did not make significant differences in this trait ($p<0.05$) (Fig. I. b). Similarly, IM treated seedlings exhibited the highest amount of shoot dry weight by a mean of 62.59%. The RM and UTN showed 51.32% and 51.85% increases in this trait, respectively compared with the control ($p<0.05$) (Fig. I. d). Root dry weight in IM- and RM-treated seedlings increased by 64.39% and 78%, respectively over the control while UTN did not make significant differences in this trait ($p<0.05$) (Fig. I. c).

**Photosynthetic pigments analysis**

The data on the analysis of photosynthetic pigments are depicted in (Fig. II). The results indicated that the amounts of photosynthetic pigments including Chlorophyll *a*, Chlorophyll *b*, and total Chlorophyll increased under the music stimulation. IM and RM led to average elevations of the level of Chlorophyll *a* by 36.6% and 31.93%, respectively compared to the control while UTN showed 19.66% increase, which did not show a significant change as compared with the control ($p<0.05$). RM exhibited the most prominent effect on the content of Chlorophyll *b* (mean 37.46%) whereas IM and UTN slightly increased this trait, not showing significant differences from the control ($p<0.05$). Similarly, the maximum total Chlorophyll increase was observed in RM-treated seedlings (mean 33.66%) and IM by an average of 31.23% compared to the control treatment. Additionally, music treatments caused a significant boost in carotenoid content (Fig. II. d), and with a similar trend, the highest increase in carotenoid content was observed in RM (mean 38%), and IM resulted in 35% increase in this trait over the control treatment. On the other hand, UTN treatment did not make a significant difference in these traits ($p<0.05$).

### *Biochemical assay analysis*

Results showed that sound treatments significantly enhanced the POD activity in leaves of 30-day-old seedlings by 53%, 57%, and 46% in IM, RM, and UTN respectively as compared with the control (Fig. III. a). Moreover, IM, RM, and UTN increased CAT activity by 31%, 25%, and 7.8% in comparison with the control (Fig III. b). Total soluble protein concentration in UTN-treated seedlings increased by mean 23%, whereas in plants treated with IM the amount of protein changed only by 4%, and enhanced over the control (Fig III. c). Interestingly, the highest concentration of proline was observed in UTN (mean 66 %), and in RM it reached 39% compared to the control. On the other hand, IM treatment only rose the proline content by 18%, which was significant at $p<0.05$, (Fig. III. d). Surprisingly, the amount of total sugar reduced in treatment groups. Among different treatments, RM showed the lowest sugar content with approximately 33.25% reduction, followed by IM and UTN with 32.27% and 24.63% reductions, respectively (Fig. III. e). Fig. (IV) shows a heatmap correlation matrix among various physiological traits evaluated in the study. According to Fig. (IV), there is a significant

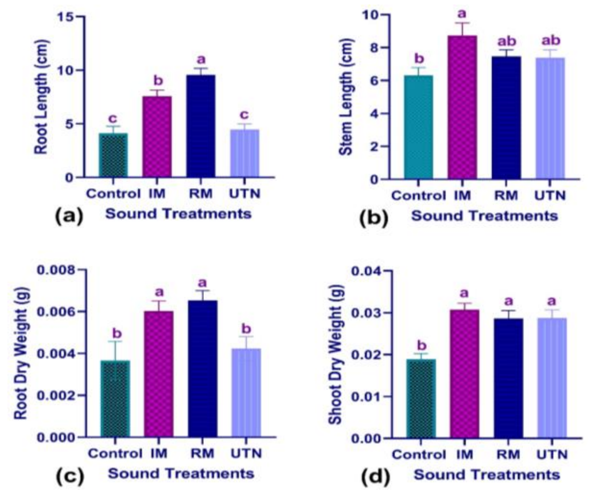


Fig. I. The effect of music and sound treatments on *S.hortensis* (30-day-old seedlings); treatments included Control, IM (Iranian Music), RM (Rock Music), and UTN (Urban/Traffic Noise). (a) Root length changes under sound treatments; (b) Shoot length changes; (c) Root dry weight; (d) Shoot dry weight; different letters on columns express significant statistical differences based on Tukey's test at $p<0.05$.

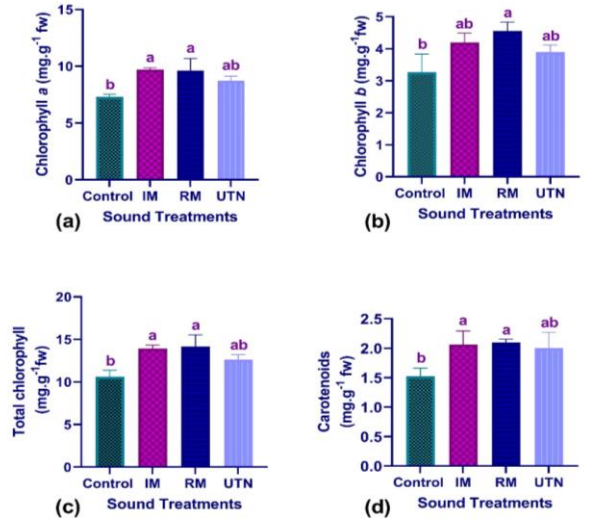


Fig. II. Changes in photosynthetic pigments of *S. hortensis* leaves under music and sound treatments; treatments included Control, IM (Iranian Music), RM (Rock Music), and UTN (Urban/Traffic Noise). (a) Chlorophyll *a*; (b) Chlorophyll *b*; (c)Total chlorophyll, and (d) Carotenoids concentration; different letters on columns express significant differences according to Tukey's test at $p<0.05$.

correlation among the monitored characteristics in the recent study.

## Discussion

The results of the present study revealed that music and sound considerably influenced the growth and development of *S. hortensis*, in terms

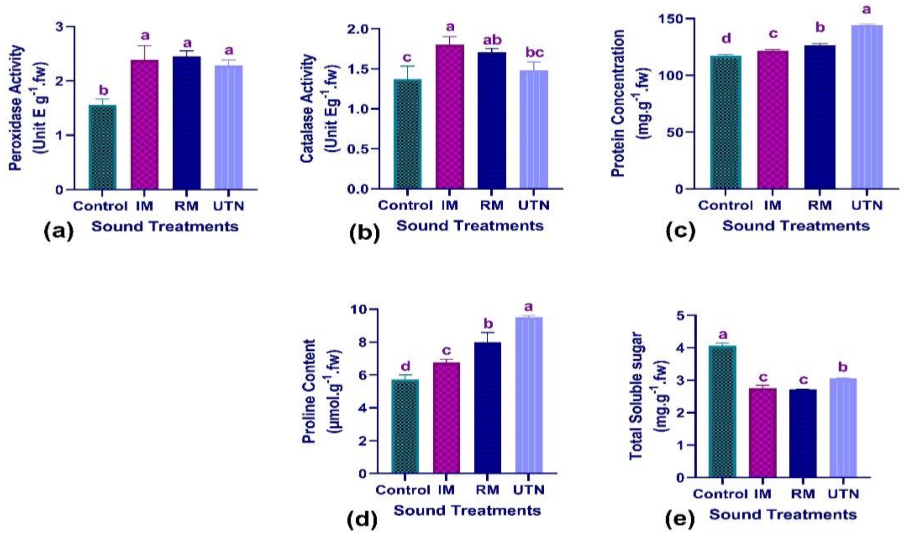


Fig. III. Biochemical changes in seedling leaves under sound treatments; treatments included Control, IM (Iranian Music), RM (Rock Music), and UTN (Urban/Traffic Noise). (a) Peroxidase enzyme activity; (b) Catalase activity; (c) Protein concentration; (d) Proline content; (e) Total soluble sugar; different letters on columns express significant differences based on Tukey's test at p<0.05.

of seedlings length and biomass. Moreover, physiological behaviors changed under sound stimulation. Sound as an external stimulus that carries on mechanical energy can trigger various biological processes (Ghosh et al., 2016; Ramekar and Gurjar, 2016). Our data confirmed that exposure to music significantly increased the content of photosynthetic pigments. The results are in agreement with HE et al. (2012) on *Rosmarinus officinalis*, who found that different types of music prompted better growth of the seedlings and increased the content of photosynthetic pigments under the music treatment.

In this study, applying music and sound accelerated the activity of peroxidase (POD) and catalase (CAT), which are two main enzymes in plants' antioxidant system (Abedi et al., 2020). Accumulation of $H_2O_2$ occurs in cells under abiotic stress, and various antioxidant enzymes such as CAT and POD increase in these situations. It is believed that $H_2O_2$ can initiate signaling pathways (Li et al. 2008).

Furthermore, soluble protein content can elevate cell division and alter the content of enzymes and relative metabolic rate (Bochu et al., 2003; Chowdhury and Gupta, 2015; Yiyao et al., 2002), and subsequent growth in plant tissues are

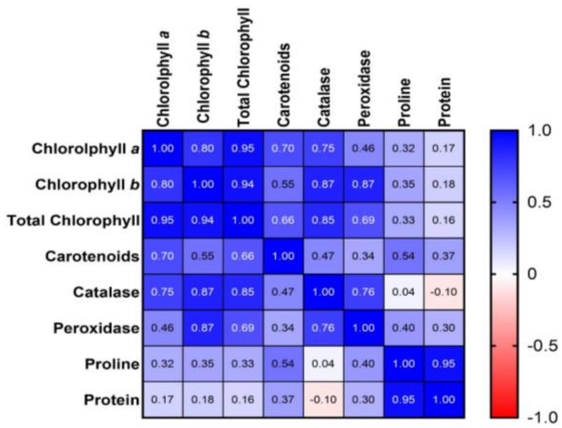


Fig. IV. Heatmap correlation matrix among different biophysiological traits evaluated in the study; levels of blue color indicate significant in correlation.

affected by soluble proteins. Hassanien (2014) demonstrated that treatment with 300-6000 Hz sound waves in *Oryza sativa* enhanced the protein content by 8.8%, and also the quality of the production improved (Hassanien et al., 2014).

Our findings also showed that proline concentration increased in the treated seedlings. A high level of proline accumulation is a common physiological reaction in plants exposed to abiotic stresses. This finding confirms that stimulation of sound as alternating stress provokes the defense

Table 1
Characteristics of the music and sound treatments

| Treatment | Iranian Music (IM) | Rock Music (RM) | Urban Traffic Noise (UTN) |
|---|---|---|---|
| Sound type | Harmonic and rhythmic | Non-Harmonic, but rhythmic | Non-Harmonic and non-rhythmic |
| Frequency Analysis Range (Hz) | 800-2000 Hz | 1100-7000 Hz | 800-2000 Hz |
| Sound Pressure Level (dB) | ~ 80 dB | ~ 80 dB | ~ 80 dB |

system in the early stages of seedling and promotes the better growth progression of *S. hortensis* plants.

In this research, soluble sugar significantly decreased in seedlings, revealing better growth indices while some previous studies implied enhancement in sugar content after sound treatments (Chowdhury and Gupta, 2015; Jeong et al., 2014). High amounts of sugar can induce inhibitory effects on photosynthesis, stunt plant growth, and lead to necrotic leaves under abiotic stress conditions. However, lower concentrations of sugars reverse all these physiological parameters (Sami et al., 2016). Taking these outcomes into account confirms that music treatments have apparent effects on *S. hortensis* growth and also differential biochemical behaviors. Nevertheless, there are some differences between treatment groups' responses that need to be considered.

According to the earlier research, the differences in sound treatments are most likely due to the frequencies and sound pressure level in each music or sound treatment (Chowdhury et al., 2014); therefore, the physiological responses in certain traits are varied. This study was considered with two accessible and magnitudes of the sound (the frequency and the sound pressure level) to understand how music could affect *S. hortensis* seedlings. The procedure was taken by analyzing the music and sound treatments by the sound meter application (Table 1). Analysis of the average frequencies of each treatment showed that IM (harmonic and rhythmic music sound) frequency is around 800-2000 Hz; RM (non-harmonic, but rhythmic sound) showed an upper-frequency range around 1100-7000 Hz while UTN (non-harmonic and non-rhythmic sound) with the most repetitive frequency is near 800-2000 Hz.

Surprisingly, our results concluded that IM and UTN samples showed the most differences in behaviors, and IM and RM with different frequency ranges, exhibited the most similarity. On the other hand, although these results suggest that the frequency and intensity of the applied sound are the main parameters in generating responses, probably other factors cannot be neglected.

Several lines of studies reported that plants can receive, perceive, and respond to various environmental stimuli. Most likely, plants are somehow able to distinguish between sounds (harmonic or non-harmonic) which raises the question of whether the plants can identify music from the noise. Vanol and Vaidya (2014) reported the positive effect of classical and rock music as opposed to the traffic noise with deterrent effect on the growth parameters of Guar plants. In contrast with their results, UTN did not exhibited inhibitory effects at the early developmental stage.

In plants without any auditory cells, the sound stimulation sensation connected to vibrations occurs at the cell surface. Several years of studies indicated that plant cells are equipped with two types of immune receptors. By these, plants can distinguish varieties of extracellular signals, for example, pathogens and many different abiotic stresses such as drought, salinity, and extra cold. They participate in mediate protein phosphorylation to integrate many internal and external signals and control the developmental processes (Cheung et al., 2020). Meanwhile, some pieces of evidence indicate that plants have selective sensitivity to the frequencies (Gagliano et al., 2017; Simon et al., 2019; Appel and Cocroft, 2014). This evidence proved that plants intelligently understand the environment and respond appropriately.

Although in some previous studies, the optimal frequency was suggested about 1000 Hz (Bochu et al., 2001; Li. et al., 2008; Yang et al., 2002), some other scientists suggested a wide range of sound waves as growth stimulants (Cai et al., 2014; Pujiwati et al., 2018; Qin et al., 2003). Overall, these findings suggest that not only responses to the sound stimulation are selective in plants, but also the optimal frequencies are dependent on the plant species and maybe several other undetermined factors. Thus, intercellular and intracellular events need to be understood.

## Conclusion

This study provides a comparative evidence on how plant responses to music can be varied from that noise. Both music and traffic noise treatments improved growth, activated the ROS-scavenging system, and stimulated secondary metabolism. However, the beneficial effects of IM (harmonic and rhythmic music) and RM (non-harmonic but rhythmic sound) on *S. hortensis* seedlings were more observed. It seems that there is a preference for the sound in plants, and based on the results of this study, it can be hypothesized that there exists a diagnostic system in plants that identify non-rhythmic sound frequency from a harmonious melody. This study also underlines the necessity of paying special attention to physical characteristics of sound, like frequency and intensity to clarify the involved mechanisms in plant/music/noise interaction. Transient traffic noise treatment was not associated with growth inhibition. However, long-time traffic noise may adversely affect plant growth and immunity, which therefore, needs to be explored in future studies.

## Acknowledgments

The authors would like to thank Dr. Soheil Mansouri for his valuable recommendations and constructive suggestions during the development of this research report.

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