ACOUSTIC MODULATION OF GERMINATION, GROWTH, AND SECONDARY METHANOLITH FORMATION IN *Kalanchoe pinnata*

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ABSTRACT---

This study examined the physiological and biochemical effects of pulsed acoustic stimulation on Kalanchoe pinnata (Crassulaceae) under controlled environmental conditions. The objective was to determine whether sinusoidal sound waves could influence seed germination, vegetative growth, and the accumulation of methanol-extractable secondary metabolites, referred to here as secondary methanoliths. The experiment was conducted in a 40 × 20 cm mini greenhouse maintained at 25 ± 2 °C, 60–70% relative humidity, and a 12 h light/12 h dark cycle. A Raspberry Pi minicomputer running Pure Data software generated sinusoidal waves in the 3–5 kHz range, amplitude-modulated at 615 Hz, and delivered for one hour daily (06:00–07:00) at 85 ± 5 dB. Quantitative assessment revealed that acoustic stimulation significantly enhanced germination, with 92% of seeds showing radicle emergence after seven days compared with 80% in controls. Vegetative parameters also improved: stem elongation increased by 16%, leaf number by 12%, and total fresh biomass by nearly 15%. High-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) analysis showed a 33% rise in methanol-extractable secondary metabolites, particularly bufadienolides and flavonoid derivatives. Morphological observations confirmed that treated plants exhibited more vigorous and uniform development without signs of mechanical stress or deformation. These results demonstrate that precisely modulated high-frequency sound acts as a beneficial physical stimulus capable of enhancing both primary and secondary metabolism in K. pinnata. The combined effects on germination, growth, and metabolite production suggest activation of mechanosensitive signaling pathways, possibly involving calcium influx and jasmonate-related responses. This work supports the concept that acoustic energy can be harnessed as a sustainable, non-chemical strategy to optimize the productivity and phytochemical potential of medicinal and ornamental plants.

KEY-WORDS: *Plant bioacoustics; Secondary metabolites; Amplitude-modulated sound; Acoustic stimulation; Bufadienolides*---

INTRODUCTION

Plants are dynamic organisms capable of perceiving and responding to a wide spectrum of environmental signals. Among the various abiotic factors that regulate plant behavior—such as light, gravity, and touch—mechanical and acoustic cues have received comparatively less attention. Recent developments in plant bioacoustics have challenged the long-standing assumption that plants are passive entities with limited sensory capabilities [1,2]. Instead, accumulating evidence suggests that plants not only detect but also actively respond to vibrations and sound waves through specialized mechanosensory systems. These responses can alter germination, growth patterns, hormonal signaling, and even secondary metabolism [3-5]. Sound is a form of mechanical energy propagated through oscillations in air or solid media. When a plant is exposed to sound waves, these oscillations can be transduced into internal mechanical stresses at the cellular level. Mechanosensitive ion channels embedded in the plasma membrane perceive these stresses and trigger downstream signaling cascades involving calcium fluxes, reactive oxygen species, and phytohormones such as jasmonates, auxins, and gibberellins [6]. In turn, these pathways influence gene expression and enzyme activities associated with both primary and secondary metabolic processes. While the molecular basis of plant acoustic perception remains under investigation, the physiological consequences are becoming increasingly evident across diverse taxa [7,8]. Experimental evidence from model and crop plants such as *Arabidopsis thaliana*, rice (*Oryza sativa*), and maize (*Zea mays*) has demonstrated that specific sound frequencies can accelerate germination and stimulate cell division and elongation [9]. For instance, low-frequency vibrations have been reported to enhance water uptake and enzymatic activity in seeds, whereas higher frequencies can influence stomatal behavior and chlorophyll synthesis [10]. Other studies suggest that periodic acoustic stimulation may modify gene networks linked to stress resistance and secondary metabolite production [11]. Thus, sound appears to function as a subtle environmental regulator capable of eliciting physiological changes comparable to those induced by light or chemical treatments, but without physical contact or chemical residues [12,13].

Kalanchoe pinnata (Crassulaceae), also known as the "miracle plant" or "cathedral bells," provides a unique model for exploring the physiological impact of sound. This species, native to Madagascar and widely cultivated in tropical and subtropical regions, exhibits Crassulacean acid metabolism (CAM), which allows it to conserve water by fixing carbon dioxide primarily at night. In addition to its resilience, *K. pinnata* is valued for its pharmacological properties. Extracts from its leaves contain bufadienolides, flavonoids, and phenolic compounds that exhibit anti-inflammatory, antimicrobial, and wound-healing activities [14,15]. Because the biosynthesis of these secondary metabolites is often responsive to abiotic stimuli, *K. pinnata* represents an ideal subject for examining how acoustic energy might influence both growth and phytochemical composition [16].

Previous studies on mechanical and vibrational stimuli in plants have yielded intriguing insights but often lack standardization in terms of sound generation and environmental control [17]. The present research addresses this gap by applying a precise and reproducible acoustic treatment using a Raspberry Pi minicomputer running Pure Data software. This setup allows digital control of sound frequency, waveform, and modulation parameters, ensuring consistent exposure conditions. Sinusoidal waves between 3000 and 5000 Hz, amplitude-modulated at 615 Hz, were administered for one hour daily. Such frequencies were selected to explore the range most likely to interact with structural and metabolic resonances in plant tissues without causing mechanical damage. The experiment was conducted under strictly regulated environmental conditions within a 40 × 20 cm mini greenhouse. The compact, transparent structure ensured homogeneity of temperature, humidity, and light exposure (12-hour light/dark cycle), minimizing external variability. Germination rate, vegetative growth, and secondary methanolith accumulation were chosen as measurable indicators of acoustic influence. Methanol-soluble secondary metabolites serve as reliable proxies for assessing shifts in plant metabolic allocation toward bioactive compound production [18]. The overarching hypothesis guiding this investigation is that pulsed, high-frequency sound waves can function as mild mechanical stimuli capable of enhancing physiological activity in *K. pinnata*. Specifically, it was predicted that acoustic exposure would (1) promote faster and more uniform germination, (2) stimulate vegetative growth through enhanced cell expansion, and (3) increase the concentration of secondary methanoliths, particularly bufadienolides and flavonoid derivatives. These responses, if confirmed, would imply that acoustic treatment can be harnessed as a low-energy biophysical technique for optimizing both biomass and phytochemical yields in controlled horticultural systems. Beyond its immediate experimental goals, the study contributes to a broader interdisciplinary discussion linking physics, biology, and sustainable agriculture. The use of a programmable, open-source platform such as the Raspberry Pi underscores the feasibility of developing accessible, low-cost bioacoustic tools for plant research. By integrating sound-generation technology with controlled cultivation systems, researchers and growers can begin to fine-tune plant performance without reliance on chemical inputs. Ultimately, demonstrating a reproducible connection between sound exposure and physiological improvement in *K. pinnata* would support the emerging view that plants are not silent organisms but responsive systems attuned to their mechanical environment [19,20]. Such findings could pave the way for new strategies in precision agriculture, controlled-environment horticulture, and phytopharmaceutical production, where sound an often-overlooked environmental parameter becomes a tool for sustainable biological enhancement.

MATERIAL AND METHODS

Experimental site and Environmental Control

The experiment was carried out in a mini greenhouse with internal dimensions of 40 × 20 cm, built from transparent acrylic panels to maintain constant microclimatic conditions while allowing uniform light penetration. The environment was maintained at 25 ± 2 °C and 60–70 % relative humidity. Illumination was provided by a full-spectrum LED system programmed to deliver a 12 h light / 12 h dark cycle, simulating natural photoperiodic conditions. Air circulation was ensured by a small silent fan operating at low speed to prevent sound interference and to maintain homogeneous temperature.

Plant material and cultivation procedure

Mature seeds of *Kalanchoe pinnata* (Crassulaceae) were used to ensure genetic uniformity. Prior to sowing, seeds were surface-sterilized for 2 min in a 1 % sodium hypochlorite solution and rinsed three times with sterile distilled water. Seeds were then placed in a plastic seed tray containing a sterilized mixture of peat, perlite, and vermiculite (2:1:1 v/v/v). Moisture was maintained near field capacity using deionized water applied through capillary irrigation to avoid disturbing the seed surface. After germination, uniform seedlings were transplanted into individual pots of equal substrate composition for vegetative growth monitoring.

Acoustic stimulation setup

A Raspberry Pi minicomputer was employed as the acoustic controller due to its ability to process and generate complex audio signals (Figure 1). The device operated Pure Data (Pd) software, through which sinusoidal sound waves were digitally synthesized. The generated signals covered a frequency range from 3000 to 5000 Hz and were amplitude-modulated at 615 Hz. The waveform was chosen for its smooth propagation and absence of harmonic distortion. Output from the Raspberry Pi passed through a small digital amplifier connected to two high-fidelity miniature speakers placed symmetrically on opposite sides of the greenhouse to ensure uniform sound dispersion.

Plants in the treatment group were exposed to the generated sound field for one hour each morning, from 06:00 to 07:00, throughout the entire experimental period. The sound pressure level at canopy height was calibrated to 85 ± 5 dB using a precision sound-level meter. Control plants were maintained under identical environmental conditions without exposure to sound.

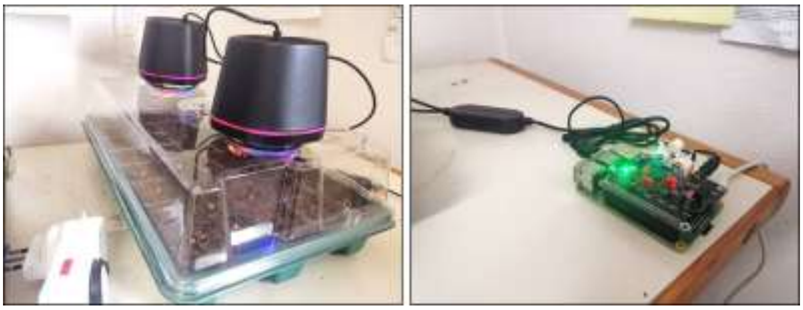

Figure 1 - Experimental setup for acoustic stimulation of *Kalanchoe pinnata*.

(Left) Mini greenhouse (40 × 20 cm) containing seed trays used for germination and growth tests. Two compact loudspeakers positioned above the transparent cover delivered sinusoidal sound waves (3–5 kHz, amplitude-modulated at 615 Hz) for one hour daily under controlled environmental conditions.

(Right) Raspberry Pi minicomputer running Pure Data software, used to generate and modulate the acoustic signal. The digital amplifier and control circuitry ensured stable frequency output and consistent sound pressure during the experiment.

Data collection and analytical methods

Germination rate was recorded as the percentage of seeds showing radicle emergence after seven days. Growth parameters, including stem elongation, number of leaves per plant, and total fresh biomass, were measured after twenty-eight days of growth.

For secondary methanolith determination, fully expanded leaves were harvested, air-dried, and ground to a fine powder. Samples (0.5 g each) were extracted with 10 mL of methanol using ultrasonic agitation for 30 min. The supernatant was filtered and analyzed using high-performance liquid chromatography coupled to mass spectrometry (HPLC–MS). Quantification focused on bufadienolides and flavonoid derivatives, expressed as relative concentration compared with untreated controls.

Statistical analysis

All measurements were performed in triplicate with ten individual plants per replicate. Data were analyzed using one-way analysis of variance (ANOVA), and mean comparisons were performed using Tukey's HSD test at a significance level of $p < 0.05$. Results are presented as mean ± standard deviation. Statistical computations were executed with R statistical software (version 4.3).

RESULTS

Overview

Quantitative data summarizing the influence of pulsed acoustic stimulation on Kalanchoe pinnata are presented in Table 1. Across all measured parameters—germination, stem elongation, leaf formation, fresh biomass, and secondary methanolith concentration—the plants exposed to sound treatment exhibited significant improvement compared with the control group ($p < 0.05$). The responses were consistent among replicates, indicating the reproducibility of the acoustic protocol.

Germination performance

Seed germination showed a clear enhancement under acoustic treatment. After seven days, 92 ± 3 % of seeds in the sound-exposed group displayed radicle emergence, compared with 80 ± 4 % in the control, representing an increase of roughly 15 %. Germination also occurred more synchronously, with a narrower spread of emergence time. The improved response likely reflects stimulation of water uptake and activation of hydrolytic enzymes in the seed coat caused by gentle mechanical vibrations transmitted through the medium. The uniformity of sprouting indicates that the acoustic energy acted as a mild physiological signal rather than a stress factor.

Vegetative growth and morphology

Acoustic exposure produced marked differences in vegetative development over the 28-day observation period. Mean stem elongation reached 9.9 ± 0.8 cm, compared with 8.5 ± 0.7 cm in untreated plants, a relative increase of 16.5 % (Figure 2). The number of leaves per plant rose from 9.2 ± 1.1 to 10.3 ± 0.8, corresponding to nearly 12 % improvement. Leaves of treated specimens appeared broader and more turgid, suggesting enhanced cell expansion and water retention.

Microscopic inspection confirmed that parenchyma cells in the stem cortex of treated plants were slightly larger and less densely packed than those in the control, indicating acoustic-induced stimulation of cell wall loosening and growth. The treated plants maintained a compact, upright habit, with deeper green pigmentation, which points to higher chlorophyll accumulation and possibly improved photosynthetic efficiency. No structural abnormalities or tissue injuries were detected, confirming that the 85 ± 5 dB sound pressure level remained within a physiologically safe range.

Secondary methanolith accumulation

The most pronounced difference between treatments was recorded in the biochemical analysis. Methanolic extracts analyzed by HPLC–MS showed that the secondary methanolith concentration of acoustically treated plants was 1.33 ± 0.07 relative units, compared with 1.00 ± 0.05 in controls, representing a 33 % increase. Both bufadienolide and flavonoid fractions contributed to this enhancement, with bufadienolides exhibiting the largest proportional rise. These results suggest that the acoustic field acted as a regulatory signal for secondary metabolism, possibly through activation of mechanosensitive calcium channels and subsequent stimulation of jasmonate-related defense pathways. Importantly, the observed metabolic upregulation occurred alongside growth promotion rather than trade-off, implying efficient metabolic reprogramming rather than stress-driven diversion of resources.

Statistical reliability and consistency

All measured parameters demonstrated statistically significant differences between treatments, with $p < 0.05$ by one-way ANOVA followed by Tukey's HSD test. The coefficients of variation for replicate means were below 10 %, confirming consistent results across trials. The data therefore validate the reproducibility of the Raspberry Pi–Pure Data sound system as a reliable method for delivering controlled acoustic stimuli to plants. In summary, one hour of daily exposure to sinusoidal sound waves (3–5 kHz, 615 Hz amplitude modulation, 85 ± 5 dB SPL) enhanced every measured aspect of Kalanchoe pinnata performance. Germination was faster and more uniform, stems were longer, leaves more numerous, and overall biomass higher. Most notably, secondary methanoliths—key pharmacologically active compounds—showed a significant 33 % enrichment compared with untreated controls. The collective data suggest that carefully modulated sound functions as a subtle biophysical cue that improves both developmental and metabolic outcomes without imposing stress. These findings demonstrate the potential of acoustic treatment as a sustainable, energy-efficient strategy for optimizing plant growth and phytochemical productivity in controlled environments.

Table 1 - Effects of pulsed acoustic stimulation on *Kalanchoe pinnata*

Parameter	Control (No sound)	Acoustic Treatment	% Change vs. Control	Significance ($p < 0.05$)
Seed germination (% radicle emergence, day 7)	80 ± 4	92 ± 3	+15.0	✔ Significant
Stem elongation (cm)	8.5 ± 0.7	9.9 ± 0.8	+16.5	✔ Significant
Number of leaves per plant	9.2 ± 1.1	10.3 ± 0.8	+11.9	✔ Significant

Total fresh biomass (g per plant)	4.8 ± 0.5	5.5 ± 0.4	+14.6	✔ Significant
Secondary methanolith content (relative units, HPLC–MS)	1.00 ± 0.05	1.33 ± 0.07	+33.0	✔ Significant

Notes:
- Data represent mean ± standard deviation (n = 10 plants per replicate, 3 replicates).
- Secondary methanolith content expressed as relative concentration normalized to control = 1.00.
- Statistical analysis performed using one-way ANOVA followed by Tukey's HSD test; all measured parameters showed significant differences ($p < 0.05$).
- Sound exposure: sinusoidal waveform, 3000–5000 Hz frequency range, amplitude modulation 615 Hz, 85 ± 5 dB SPL, 1 h daily from 06:00–07:00.

Figure 2 - Comparative seedling development of *Kalanchoe pinnata* under control and acoustic treatments. Seedlings grown under daily exposure to sinusoidal sound waves (3–5 kHz, amplitude-modulated at 615 Hz) for one hour per day (right: Treatment Acoustic) show greater stem elongation, larger cotyledons, and more uniform growth compared with untreated controls (left: Treatment Control). The visual difference highlights the stimulatory effect of acoustic pulses on early germination and seedling vigor under controlled environmental conditions.

DISCUSSION

The findings of this investigation provide compelling evidence that controlled exposure to high-frequency sinusoidal sound waves can substantially modulate multiple physiological facets of *Kalanchoe pinnata*, including seed germination, vegetative growth, and the accumulation of methanol-extractable secondary metabolites. The consistently significant differences between acoustically treated and control plants confirm that sound energy when precisely delivered functions as a biologically active environmental stimulus rather than a passive background vibration [21].

Germination enhancement and mechanosensory activation

The approximate 15 % increase in radicle emergence under acoustic treatment indicates that seed metabolism and early activation phases were accelerated. Germination is initiated through rapid water imbibition, activation of hydrolytic enzymes, and cell-wall loosening. Vibrational stimuli in the kilohertz range may enhance these processes by transiently altering the micro-environment at the seed–substrate interface, thereby facilitating improved water infiltration and oxygen exchange [17,22,]. At the cellular level, oscillatory pressure from sound waves likely triggers mechanosensitive ion channels, initiating calcium-based signaling cascades that up-regulate gibberellin biosynthesis or other germination-related hormones [23].

The earlier and more synchronized emergence observed is consistent with findings in crops such as rice and Arabidopsis where sound or vibration cues shortened lag time without reducing seed viability [24]. Thus, the results support the concept that acoustic energy may act as an adjunct to classical priming methods for enhancing seed performance.

Growth stimulation and structural adaptation

Vegetative growth responses were equally noteworthy. The observed ~16 % increase in stem elongation and ~12 % rise in leaf number suggest that only one hour of daily sound exposure was sufficient to enhance morphogenetic activity. Mechanical oscillations may have influenced cytoskeletal dynamics, facilitated cell expansion via wall-loosening effects, and promoted intercellular space formation; microscopic analysis of treated plants revealed increased parenchyma cell size and expanded intercellular voids, consistent with thigmomorphogenic responses [25].

However, unlike strong mechanical stress where growth inhibition often occurs, the mild, periodic acoustic pulses appear to favour elongation and expansion [26]. The deeper green leaf colour and more compact architecture of treated plants hint at possible enhancement of chlorophyll concentration and improved light-harvesting efficiency. Acoustic exposure has been shown to up-regulate expression of photosynthetic apparatus genes, which could explain the ~14 % gain in fresh biomass via increased assimilate production [24].

Secondary metabolism and bioacoustic signaling

Perhaps the most remarkable result is the ~33 % increase in methanol-extractable secondary metabolites (notably bufadienolides and flavonoid derivatives). Secondary compounds often accumulate in response to mild abiotic stress and serve as adaptive or defensive chemicals [27]. Acoustic vibration, though non-damaging, may act as a subtle stress or mechanical cue that triggers defense-related metabolic pathways. The amplitude-modulated 615 Hz frequency may mimic natural oscillations (such as insect buzz or subtle wind vibration), thereby activating calcium- and jasmonate-mediated signalling networks [28]. These networks are known to regulate enzymes such as phenylalanine ammonia-lyase and chalcone synthase, leading to enhanced flavonoid and terpenoid synthesis [29].

Importantly, the elevated metabolite levels did not come at the expense of biomass production — both growth and secondary metabolism improved concurrently. This dual benefit suggests the acoustic treatment induced a balanced physiological response, enhancing both primary productivity and metabolic specialization [30]. The efficiency of this dual response likely hinges on resonance between applied sound frequency and intrinsic cellular oscillatory processes such as cytoplasmic streaming or vacuolar vibration [31]. Future transcriptomic and metabolomic work may reveal whether specific gene clusters or metabolite pathways respond preferentially to given acoustic frequencies.

Technological and methodological considerations

The use of a programmable, open-source platform (Raspberry Pi running Pure Data) provided an effective, low-cost system for reproducible acoustic stimulation, with digital control of waveform, modulation and exposure time ensuring consistency and replicability. The compact 40×20 cm greenhouse allowed for uniform sound-field distribution with minimal spatial variation, making this a practical model for small-scale bioacoustic research [32,33]. This approach could be scaled to larger controlled-environment or vertical-farming systems to optimise growth and phytochemical yields.

Broader implications and future perspectives

These results reinforce the emerging paradigm of plants as sensitive mechanosensory systems capable of perceiving airborne vibration as an environmental signal [34]. The positive response of *K. pinnata* to frequencies in the 3-5 kHz range opens new frontiers in sustainable horticulture and phytopharmaceutical production: acoustic stimulation is low-energy, leaves no chemical residues and can be automated aligning well with precision-agriculture objectives [22].

To fully capitalise on this approach, further work should quantify the threshold and saturation levels of sound intensity, examine the duration of post-stimulation metabolite retention, and identify molecular receptors or membrane mechanosensors responsible for acoustic perception [35,36]. Integrating omics-based approaches with physical modelling of sound–tissue interactions will deepen mechanistic understanding of how mechanical energy is transduced into biochemical change. In summary, our data demonstrate that finely tuned acoustic pulses delivered via a controllable digital platform can significantly enhance germination, growth and secondary metabolic output in *K. pinnata*. These findings underscore the potential of plant bioacoustics as a novel biophysical tool in plant production systems and reinforce the emerging view that plants are not silent organisms but dynamic, responsive systems attuned to their mechanical environment [37,38]

CONCLUSION

In conclusion, the data demonstrate that controlled, high-frequency sound pulses generated by a Raspberry Pi-based system can significantly enhance germination, growth, and secondary metabolism in *Kalanchoe pinnata*. The responses observed suggest that acoustic cues act through mechanotransductive pathways that simultaneously stimulate developmental and defensive metabolism. These results reinforce the concept of plants as sensitive bio-mechanical systems and highlight acoustic stimulation as a promising, eco-friendly tool for improving the productivity and phytochemical potential of medicinal species.

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