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Can music invoke defence response in plants ?

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Plants are complex multicellular organisms that can not only sense plethora a of natural sounds in the surrounding environment, but can also respond and communicate with the sound vibrations (SV) perceived from above and under the ground. In the present investigation an attempt has been made to explore the role of SV in the form of Indian classical music in triggering defence response in periwinkle (*Catharanthus roseus*(L.) G.Don) against leaf spot disease caused by fungal pathogen, *Alternaria alternata*. Two different Ragas of Indian classical music,Rag Hansadhwani (M1) and Rag Darbari Kanada (M2)were used for treatment. In the pilot study, it was observed that both the music treatment showed a positive effect on growth of mung bean seedling. Further studies with periwinkle revealed that music treatment can cause significant increase inphenol content and defence related enzyme activities in the leaves of treated plants. A positive correlation was observed between the complexity of Raga (determined by its W value) and its effect on plant metabolism. Darbari Kanada with much higher W value also exhibited a greater effect thanHansadhwani in elicitation of plant defence as manifested by significant increase in total phenol, Peroxidase (PO), Polyphenol oxidase (PPO), Phenylalanine ammonia Lyase (PAL) and β-1,3-glucanase activity. Results of pathogenicity test also showed greater reduction of leaf spot disease on detached leaves in case of M_2 treated plants (72%) than M_1 treatment (64%). Present investigation revealed that music intervention may activate defence mechanism in plants and this finding indicates a potential new avenue in the field of plant disease management.

Keywords : *Catharanthus roseus*;defence enzymes; elicitation; Indian classical music**;** leaf spot disease

INTRODUCTION

Sound is defined as a vibration that can propagate as an audible wave of pressure through medium of solid, liquid or gas (Bhandawat and Jayswal, 2022; Jung *et al.* 2018). The three parameters by which sound waves can be characterized are frequency (Hertz or Hz), intensity (Decibel or dB) and timbre (Frongia *et al.* 2020). On the basis of frequency bands Rosen *et al.* (2011) divided naturally audible sound intolow-frequency bands (20 Hz to 200 Hz); medium-low frequency bands (200 Hz to 1 kHz), medium-high frequency bands (1 kHz to 5 kHz) and high-frequency bands (5 kHz to 20 kHz). Sound with frequency band > 20 kHz is considered as ultrasonic and < 20 Hz as infrasonic (Bhandawat and Jayswall, 2022).

Plants live amidst different types of natural sounds like flowing water, rain fall, wind, bird's chirp, bee's buzz andsounds made by insects and other herbivores. This type of natural music is referred as "green music" (Ghosh *et al.* 2017). Plants are considered to be sensitive inassaying the effects of sounds and they can also respond to music in the same way as humans do (Benford*,* 2002). However, like human and other animals the mechanism of sound perception by plants is not yet understood clearly.This is also noteworthy thatnot only plants can perceive sound but they can also emit a wide variety of sounds having different frequencies and intensities and thus can communicate with world above and under the ground (Stabile *et al.* 2022). Khait *et al.*(2023) recorded sounds emitted in the ultrasonic range of 20-100 kHz by tomato and tobacco plants inside acoustic chamber and in a greenhouse under drought stress and injured condition. Earlier

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researches haveestablished that SV has a positive impact on growth and development of plants. Green music exhibited positive effect on seed germination, growth, yield, chlorophyll and starch content and stress tolerance in a number of plant species (Akhoundnejad 2019; Bhandawatand Jayswal 2022; Hendrawan *et al.* 2020; Qin *et al.* 2003; Sharma *et al.* 2015). Apart from growth parameters, sound can also trigger expression of vital genes involved in plant hormone synthesis, protective enzyme activity and plant immune response (Ghosh *et al.* 2016). Safari *et al.* (2013) revealed that in *Corylus avellana* cells, expression of gene encoding catalase (CAT) and phenylalanine ammonia lyase (PAL) increased when treated with low intensity ultrasound. Jaramillo (2018) also reported that SV can trigger Ca^{2+} and ROS signalling pathways in stressed plant cells. Azgomi *et al.* (2023) observed that music treatments including Iranian music, rock music and urban traffic noise can stimulate germination, growth and biomass accumulation in *Satureja hortensis* (L.). The low and high frequency treatment also increased PAL and total phenol concentration. They also noted that plants responded differently on rhythmic and non-rhythmic sounds which is manifested by their physiological and biochemical metabolism. There are earlier reports that Plant Acoustic Frequency Technology or PAFT has been employed to agriculture field and an increase in number of flowers, fruits, leaves and chlorophyll content has been observed in lettuce, strawberry, sweet paper, and tomato plants (Fan *et al.* 2010; Hassanien *et al.* 2014; Meng *et al.* 2011,2012). It has been also reported that sonic exposure activates innate immunity in plants. SV mediated plant protection was observed through the activation of systematic immune response in crop plants such as pepper, cucumber, melon and strawberry (Chowdhury *et al.* 2014; Hou *et al.* 2014,; Mishra *et al.* 2016).

Apart from natural green music,ethnic traditional music can also stimulate the growth and metabolic activities of plants. Hendrawan *et al.*(2020) showed that treatment of Chinese broccoli plants with Javanese musicresulted a positive impact on growth parameters of plant.Similarly telegraph plantsand rice when exposed to Buddhist chants showed better growth and yield (Munasinghe *et al.* 2018; Stabileet *al.* 2022). Chevkula (2014) revealed that *Rosa sinensis* plants when exposed to Vedic chants showed maximum shoot elongation and increase in number of flowers. On the other hand the plants treated with Indian classical music showed highest inter node elongation.

Current investigation mainly focuses on the role of sonic exposure in activation ofdefence response in periwinkleagainst leaf spot disease caused by *Alternaria alternata*. Herean attempt has been taken to explore theeffects of two ragas of Indian classical music in elicitation of plant immune response against fungal pathogen.

MATERIALS AND METHODS

Plant material and Fungal culture

The experiments were carried out with periwinkleplants in the experimental garden of Scottish Church College. The seeds were sown in soil (clay: sand: vermicompost in 3:1:1 v/v) taken in earthenware pots. To ensure proper maintenance of plants, they were watered regularly. For the experiments with mung bean seedlings, seeds were germinated and were sown in the soil taken in earthenware pots. Five-day old seedlings were taken for music treatment.Five-day old seedlings were taken for music treatment. The culture of pathogenic fungus *Alternaria alternata* was obtained from Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta and was maintained in Potato dextrose agar (PDA) medium.

Music treatment and music analysis

One month old plants were treated with sound of two different Ragas of Indian classical music, Rag Hansadhwani (M1) and Rag Darbari Kanada (M2). Music treatment was carried out in a completely soundproof chamber where the sound of external environment is not present. One month old plants were exposed to music for one hour twice daily. The treatment was carried out for 28 days respectively. Same music treatment was applied to the seedlings of mung bean seedlings for 14 days once daily for an hour. Sonic exposure was given to the plants in instrumental form in

Sarod recital.The experiments were carried out in separate sound proof chambers to avoid any kind of random errors. Control condition was maintained by keeping the plants in sound free room and other two sets of plants were treated with music respectively. The same process was followed both in case ofperiwinkleandmung bean seedlings.

Both the ragas were characterised by W (Multifractal spectral width) value which indicates the width that is the degree of complexity of the particular music sample. Music is a complex system consisting of function of frequency, amplitude and timbre. Here, chaos based non-linear approach was used to quantify the degree of complexity of each music sample and the multifractal spectral width was analyzed by using multifractal detrended fluctuation analysis (Basu *et al.* 2023)

***In vitro* assays of total phenol and defence enzymes after music treatment**

Total phenol

Total phenol content was estimated by following the method of Paul *et al.* (2023). Phenol extraction was done in 80% methanol and absorbance was measured at 725 nm. Phenol content was calculated as mg gallic acid equivalents g^{-1} leaf tissue.

Defence relatedEnzyme assays

For PPO and PO assay, leaf tissues (200mg) were collected from treated and control plants and extractionwas done in 2 ml of 0.1 M of sodium phosphate buffer (pH 7.0) with 0.1% polyvinylpyrrolidone and 20 µL of 1 mM phenylmethylsulphonyl fluoride. Sodium acetate buffer (0.1M, pH 5.0) and sodium borate buffer (0.1 M, pH 8.7) were used for β-1,3-glucanase and PAL extraction. The extraction procedures were conducted at 4° C and homogenate was allowed to centrifuge at 12,000×g for 20 min at 4° C. The supernatant obtained was used as crude enzyme source (Chakraborty *et al.* 2017, 2019).To calculate the enzyme activity in respect to protein, total protein was estimated by standard Bradford assay (Bradford 1976) using Bovine serum albumin (BSA) as a standard.

PO assay was carried out following the method of Hemeda and Klein (1990) and Paul *et al.* (2023). Reaction mixture was prepared by adding 0.05 ml enzyme extract with 2.95 ml of substrate (5 ml of 0.1% guaiacol, 5 ml of 0.3% H_2O_2 and 50 ml of 0.1 M sodium phosphate buffer of pH 6.5). The absorption changes were recorded for 0-3 mins at 30 s of interval in UV–Vis spectrophotometer at 470 nm. Enzyme activity was calculated as µmol min^{-1} mg^{-1} of protein (E=26.6 mM^{-1} cm^{-1}).

Activity of PPO was estimated following the method of Kumar and Khan (1982) and Paul *et al.* (2023). The reaction mixture was prepared by adding 0.1 M catechol (1.4 ml), 0.1 M sodium phosphate buffer (pH 6.5) (1.6 ml) and crude enzyme extract (0.5ml). After incubating the reaction mixture for 30 min at room temperature, the enzymatic reaction was stopped by adding 2.5 N H_2SO_4(1ml). Absorption was measured in UV–Vis spectrophotometer at 495 nm against a blank set without enzyme. The PPO activity was expressed as U $min^{-1}mg^{-1}$ protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Evaluation of PAL activity was carried out following the method of Dickerson *et al.* (1984) and Paul *et al.* (2023). Here, the reaction mixture was prepared by adding 0.1 ml enzyme extract with substrate (0.5 ml of 12 mM phenyl alanine and 1.4 ml of 0.1 M borate buffer, pH 8.7). The reaction mixture was incubated for 1h at room temperature and enzyme activity was measured at 290 nm in UV-vis spectrophotometer. Enzyme activity was expressed as synthesis of trans cinnamic acid (n mol) min^{-1} mg^{-1}protein.

β-1,3-glucanase assay was done following the method of Pan *et al.* (1991) & Paul*et al.*(2023). Enzyme extract (50 µl) was mixed with an equal amount of laminarin (1%). The mixture was incubated at room temperature for 30 min. After incubation dinitro salicylic acid reagent (0.3 ml) was added to the reaction mixture and then it was boiled in water bath for 5 min.Finallythe volume of the reaction mixture was made up to 2 ml by adding distilled water. The absorption was measured at 520 nm in UV spectrophotometer against a blank set.Theenzyme activity was expressed as µmol of glucose produced min^{-1} g^{-1} protein.

Pathogenicity test to calculate the disease index on detached leaf

Leaves of control and treated plants were collected and surface sterilized. Spore suspension of *A.alternata* was put drop wise on the leaves of control and treated sets separately. The leaves were kept in a moist chamber for 48 hrs and after the incubation period disease index was noted. Percentage of disease severity was measured by the formula,

$$\text{Percentage (\%) of disease severity} = \frac{\text{No. of lesions observed} \times 100}{\text{No. of spots given}}$$

Statistical Analysis

Statistical analysis was performed using SPSS software version 20 (for one way ANOVA) and Duncan's Multiple Range test. The results are presented as mean ± standard deviation (SD). Values showing Pd<0.05 are considered as statistically significant.

RESULTS AND DISCUSSION

Music analysis

The two ragas used here are of two different genres. Hansadhwani is a popular raga in north India. It is an auspicious raga which evolves clear thinking, inner peace and happiness. On the other hand, Darbari Kanada belongs to Carnatic gharana. This raga has a profound emotional impact and brings out the feeling of deep love and pathos. The W value of M_2(1.429982) was found to be about 3-fold higher than W value of M_1 (0.498899) indicating the more complexity in M_2than M_1in terms of function of frequency, amplitude and timbre

Effect of music on plant growth

Initially a pilot experiment was designed to investigate the effect of music treatment on mung bean seedlings (4- day-old). The result showed that the shoot length of germinated seedlings increased significantly in M_1 and M_2 treated plants than the control set. Music treatment was carried out for 14 days, 1 hr daily in the morning from 8-9am (Fig. 1).After the pilot experiment music treatment was carried out in the field with periwinkle (30 days old). The results revealed no significant change in average shoot length, leaf number, bud number in plants after music treatment. The music treatment was carried out in sound proof room for 1 hr twice a day. The time for music exposure was 8-9 am in the morning and 5-6 pm in the evening. The speakers

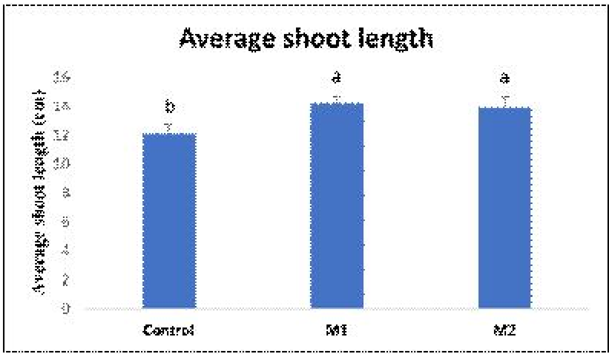

Fig 1: Effect of music on shoot length of mung bean seedlings. Values represented as mean ± SD. Significant difference in shoot length between control and music treated sets at P<0.05.

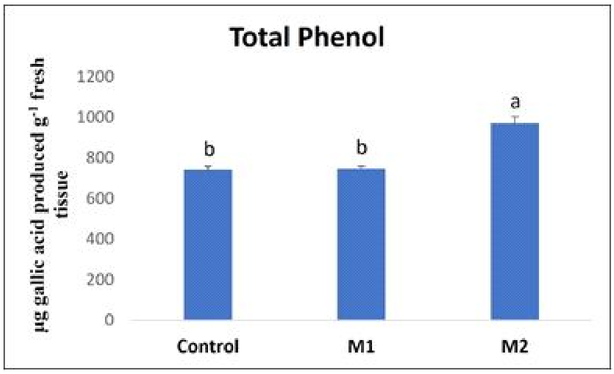

Fig 2 : Effect of music on phenol content in leaves of Periwinkle. Values represented as mean±SD. Significant difference in total phenol content between control and music treated sets at P<0.05.

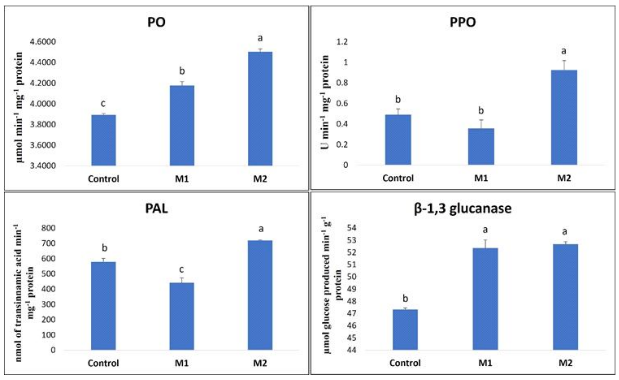

Fig. 3. Effect of music on defence enzymes in leaves of Periwinkle. Values represented as mean±SD. Significant difference in enzyme activities between control and music treated sets at P<0.05.

Table1: Effect of music treatment on disease index, total phenol and defence enzyme activities in the leaves of control and treated plants

Treatment	Disease index (%)	Total Phenol content (µg gallic acid produced/g tissue)	PO (µmol/min/mg protein)	PPO (U /min/mg protein)	PAL (nmol of transcinnamic acid/min/mg protein)	β-1,3-glucanase (µmol glucose produced/min/mg protein)
C	72.5 ± 1.56	741.90 ± 18.06	3.89 ± 0.014	0.49 ± 0.057	583.33 ± 26.62	47.39±0.06
M_1	26.25± 1.85#	747.85 ± 11.48	4.17 ± 0.038*	0.36± 0.082#	441.36± 32.26#	52.54±0.83*
M_2	20.0 ± 2.08#	970.25±33.10*	4.50 ± 0.028*	0.93 ± 0.092*	719.96 ± 3.17*	52.55±0.094*

Values represent mean ± SD of three replica
*Indicates significant up-regulation and # indicates significant down regulation with respect to control sets at P < 0.05

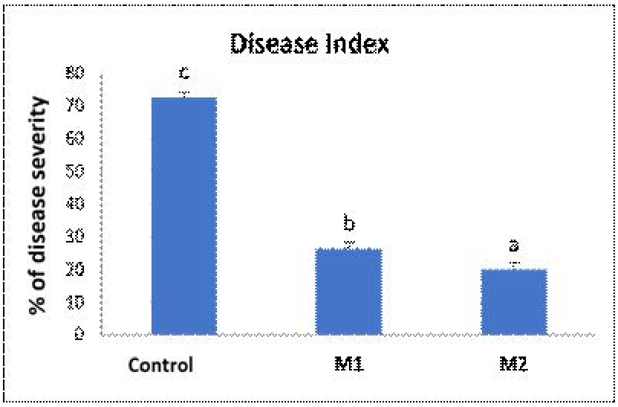

Fig. 4: Effect of music treatment on leaf spot disease of Periwinkle. Values represented as mean±SD. Significant difference in disease index between treated and control sets at P<0.05.

were kept 2 m from the plants. The shoot length was measured using measuring tape.

Effect of music on total phenol anddefence related enzyme activity

Total phenol content was found to increase significantly due to music treatment in plants. Total phenol content was found to increase (1.3-fold) significantly in M_2 treated plants. No significant change in phenol content was noted in M_1 treated plants when compared to control(Table1& Fig. 2).

Treatment with both the music ragas showed a significant upregulation in activities of defence enzymes PO, PPO, PAL and β-1,3-glucanase in the plants. Both PO and β-1,3-glucanase activity showed significant increase in leaves of M_1 and M_2 treated plants when compared to control. On contrary PAL (1.2-fold) and PPO activity (2-fold) increased only in leaves of M_2 treated plants(Table1 & Fig.3).

Effect of music treatment on leaf spot disease of Periwinkle

Results of pathogenicity test on detached leaves of both control and in M_1 and M_2 treated plants showed a significant reduction in disease with respect to control. About 64% reduction in leaf spot disease was observed in M_1 treated leaves whereas M_2 treated leaves exhibited 72% reduction in disease (Table1 & Fig.4).

Present investigation revealed a positive role of Indian classical music in induction of defence in periwinkle. Although it is still not very clearly understood how do plants perceive external sounds, but a number of studies in this field have established the fact that sounds can trigger signalling pathways in plants and through a mechanosensory machinery plants can get a number of benefits from sound (Chowdhury *et al.*2014; Gagliano *et al.*2012; Jung *et al.* 2018; Mishra *et al.* 2016).

Thus, the biological significance of sounds in plants cannot be denied. Earlier studies have clearly proved that plants can respond to the sounds of green music produced by underground flowing water, pollinators, birds and other herbivores (Appel and Cocroft,2014; Veits *et al.*2019, Wu *et al.* 2023). In the pilot experiment, it was observed that both the ragas of Indian classical music, M1 and M2 showed a positive effect on growth of mung bean seedling.Cai *et al.* (2014) also observed a similar result that sonic exposure at variable frequency exerted a positive effect on germination and growth of mung beans *in vitro* and decreased the germination period of mung beans. Further study revealed that music treatment can induce defence response in periwinkle against leaf spot disease. Significant increase in phenol content and defence related enzyme activities were noted in leaves of treated plants. The results of music analysis also showed that the two ragas used here are different in terms of their complex nature which is indicated by their W values. The W value of M_2 was found to be about 3-fold higher than W value of M_1.Thus a positive correlation was observed between the complexity of raga and their effect on plant metabolism. Darbari Kanada with much higher W value also exhibited a greater impact on plant defence as manifested by significant increase in total phenol, PO, PPO, PAL and â-1,3-glucanase activity than Hansadhwani. Results of pathogenicity test also showed greater reduction of leaf spot disease on detached leaves of M_2 treated plants (72%) than M_1 treatment (64%).

There are earlier reports that sound vibration can induce disease resistance in plants against phytopathogens. Ghosh *et al.* (2016) studied the global gene expression changes in *Arabidopsis thaliana* upon treatment of five different simple frequencies of SV at constant amplitude for 1 hr. Many defence related genes were differentially expressed after SV treatment. Phytohormonal analysis also indicated specific alterations in levels of Salicylic acid which plays a vital role in plant defence mechanism and thus can activate innate immunity in plants. Appel and Cocroft (2014) observed that sound produced during insect chewing, acts as a signal and triggers the synthesis of more defence related molecules such as glucosinolate and anthocyanin in *Arabidopsis.* Choi *et al.*(2017) showed a positive role of SV treatment (1000 Hz – 100 dB) in *A. thaliana* against *Botrytis cinerea* infection by upregulation several defence and SA responsive or signalling genes.

CONCLUSION

Present investigation highlights the event of perception of SV by plants and its potential advantageous role in triggering plant defence. From this study, it is evident that in addition to improving plant health, music treatment may activate innate immunity in plants. The results of this study open up a new avenue in the field of eco-friendly plant disease management. However, to understand the mechanism of music intervention in plant defence, more investigations are needed to be carried out to establish the use of Indian classical music in the field for plant health improvement and induction of disease resistance.

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DECLARATION

Conflict of interest. Authors declareno conflict of interest.

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