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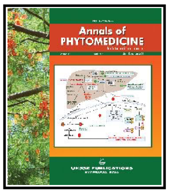

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Changes in antioxidant enzyme activity and lipid peroxidation in *Brassica juncea* (L.) Czern., by the effect of Indian classical music on the young plant stage

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Abstract

The effect of Indian classical music on the seeds of *Brassica juncea* (L.) Czern., sown in a plastic pot by using garden soil under the favourable conditions of the plant growth chamber. The influence of sound on plants can vary depending on the frequency, intensity, and duration of exposure, as seen during seed germination and seedling growth. Research attempts were made to identify the priming role of Indian classical musical vibration in favour of plant growth through its physiological parameters in six varieties (RH-406, DR-31, NRCHB-101, Pusa, Kranti, and NRCDR-2) of mustard *(B. juncea)* seedlings. The study proceeded with an effect of Indian classical music like morning meditation (MM music) and Gaytri mantra with 10 seeds of each variety of mustard under their control (without treatment), and all music nodes were applied separately. We observed that six varieties of *B. juncea* have been found to be effective with MM music. The amount of malondialdehyde (MDA) and the activity of the enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and lipid peroxidation were measured in the samples. This study indicated that antioxidant mechanisms with music exposers had a positive effect compared with those without music exposers.

1. Introduction

In the fields of plant biotechnology and plant tissue culture, soft music is a new way to promote plant growth and development, which ultimately affects the growth of yield (Callicott and Christina, 2013). Music, in today's demand, is one of the most popular, eco-friendly, and positive therapies that influences the physical and physiological conditions of not only human beings but also plants. Plants are influenced by different types of musical frequencies, either positively or negatively. In various plants, positive effects of Vedic mantras have been noticed, like positive growth of the plant's leaf size, roots, internodes, and buds (Novotney, 2013; Abdullah *et al.,* 2019). Hard-core vibration of the music may have a negative effect on plant growth, like heavy metal music, even at its low volume, which can be damaging to a sensitive plant. While devotional and soft music or classical music with their gentle vibrations positively favour the growth of plants, *i.e.,* violin music, classical ragas, *etc.* (Mishra *et al.,* 2016; Govindaraj *et al.,* 2017).

According to Fahimirad *et al.* (2013), plant cells produce oxidise free radicals such as O_2, OH, and singlet oxygen ($^1O_2$) through a variety of activities. These free radicals have a potent ability to a wide variety of useful compounds. As a result, since H_2O_2 can produce more OH and $^1O_2$ free radicals, its removal is crucial for maintaining low free radical levels in plant cells. Superoxide dismutase (SOD) removes oxygen, while peroxidase (POD) and catalase (CAT) predominantly break down H_2O_2 (Gadjev *et al.,* 2006). As a result of increased SOD, POD, and CAT activity brought on by sound wave stimulation, the formation of active oxygen species (AOS), which may shield cells from oxidative damage, was significantly reduced. Additionally, certain cell compartments may activate various defence mechanisms, directly preventing the overproduction of AOS.

Sound wave stress produces lipid peroxidation in *Dendrobium candidum* by increasing the content of malondialdehyde, a result of the breakdown of polyunsaturated fatty acid hydroperoxides (Li *et al.,* 2008). Xiaocheng *et al.* (2003) investigated the impact of sound waves on chrysanthemum production of nucleic acids and proteins. The researchers discovered that while sound waves improved RNA and soluble protein synthesis, they had no effect on DNA levels. According to the authors' findings, sound wave stimulation may activate a number of stress-induced genes, increasing transcription (López-Ribera *et al.,* 2017).

2. Materials and Methods

The experiment was performed in the plant growth chamber in the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India. Three varieties of *B. juncea* seeds (DRMRIJ-31, RH-406, NRCHB-101) and soil samples were collected from Krishi Vigyan Kendra (KVK) at Banasthali Vidyapith. While other three varieties (Pusa, Kranti, and NRCDR-2) of *B. juncea* were collected from the ICAR Directorate of Rapeseed and Mustard Research (ICAR-DRMR), Bharatpur (Rajasthan), India. The mustard seedlings were sown separately in similar plastic pots (10 seedlings in each pot) containing 500 g of soil. The clay-loam-textured soil was selected for

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this experiment. The pH of the soil was maintained at 6.5 to 7.0 (Bronick and Lal, 2005). Morning median (MM) music and Gaytri mantra music were applied by using the TTPL-USB-831 speaker.

Each variety of mustard received 10 pots, which were separated into two treatment groups and a control group. Each group was maintaining a distance of 0.20 metres by separating into plant growth chambers. The treatment groups were amplified with a TECH-COM amplifier connected to two speakers. The duration of musical treatment was constant for 6 h every day (Hussain *et al.,* 2019). Before being put into the appropriate soil pots, seeds of three different types were pre-treated by having each of the two musical nodes separately embedded on wet filter paper in petri plates for 2 h. All of the pots were exposed to 16 h of 27°C daylight and 8 h of 24°C darkness. Under continuous ambient circumstances, the entire treatment was continued for 14 days at an intensity ranging from 70 dB to 90 dB (Chowdhury and Gupta, 2015).

2.1 Meditation music morning instrumental

Flautist - Rakesh Chaurasia (Flute); sung in Chorus. 1st rag Lalit, 2nd rag Bairagi Bhairav, 3rd rag Ahir Bhairav, 4th rag Charukeshi and beats Western.

YouTube Channel name Music Today: https://youtu.be/2eLrILx3s9k

2.2 Gayatri mantra

Rag Mishra Bhupali/Deshkar

Singer - Vidushi Devaki Pandit

Album- Magic of Krishna: Sacred Chantings of Krishna

Track name - Gayatri Mantra (Classic and Soothing)- Devaki Pandit

YouTube Channel name -https://youtu.be/rYMXa8bsVPA

2.3 Estimation of antioxidant enzymes

To measure SOD activity, 0.2 g of frozen samples were homogenised in 3 ml of HEPES-KOH (pH 7.8) buffer with 0.1 mM EDTA using an ice-cold mortar and pestle. At 4^{o}C, the homogenate was centrifuged for 15 min at 15000 rpm. The SOD enzyme was obtained from the supernatant. Gianaopolitis and Reis (1977) used a photochemical technique to detect SOD activity.

In the reaction mixture (3 ml), there were the following ingredients: 0.1 mM EDTA. In the reaction mixture (3 ml), there were the following ingredients: 0.1 mM EDTA, 50 mM HEPES-KOH buffer (pH 7.8), 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 NBT, 300 l enzyme extract, and 1 mM riboflavin. The absorbance was measured at 560 nm, and one unit activity of SOD was determined as the rate of enzyme required to suppress the rate of NBT degradation by 50%. Method for measuring CAT activity was utilised as a reference (Cakmak and Horsrt, 1991).

The reaction mixture contained 40 ml of enzyme, 400 ml of 10 mM H_2O_2, and 2.6 ml of 25 mM Na-phosphate buffer (pH 6.8). The breakdown of H_2O_2 was followed by a reduction in absorbance at 240 nm. POD activity was measured in a reaction mixture containing the appropriate quantities of 28 mM guaiacol, 5 mM H_2O_2, 25 mM Na-phosphate buffer (pH 6.8), and enzyme (Ghanati *et al.,* 2002). The Bradford method was used to calculate the amount of soluble protein, and BSA was utilised as a reference (Jones *et al.,*1989).

Lipid peroxidation estimation using an MDA content metre, lipid peroxidation was calculated. 10% (v/v) trichloroacetic acid was used to homogenise the 0.2 g frozen samples in 3 ml. Each extraction received 1 ml of a 0.5% (v/v) thiobarbituric acid (TBA) solution after the homogenate had been filtered through filter paper. The final extraction was cooked for 30 min at 100°C in a boiling water bath before cooling in an ice bath. At 532 nm, the solution's absorbance was measured. This was followed by a correlation measurement of the non-specific absorbance at 600 nm. According to De Vos *et al.* (1991), who described the extinction coefficient as being 155 mM^{-1} cm^{-1}, the amount of MDA was calculated.

3. Results

3.1 Lipid peroxidation

The lipid peroxidation levels in seedlings of different varieties of *B. juncea* were determined by the treatment of two Indian classical songs as the content of MDA. The level of MDA showed variation with music treatments (Figure 1). In the morning meditation musical treatment, the level of MDA gradually increased with the effect of music in all six different varieties of *B. juncea* on the 15th day, respectively. Though, we found that six varieties of *B. juncea* have been found to be effective, Kranti and NR-2 have been reported for their better responses than the other four varieties of *B. juncea, i.e.,* DR-31, NR-101, Pusa, and RH-406. Here, we also observed that all six varieties of *B. juncea* have better responses and are slightly different from each other compared to the control.

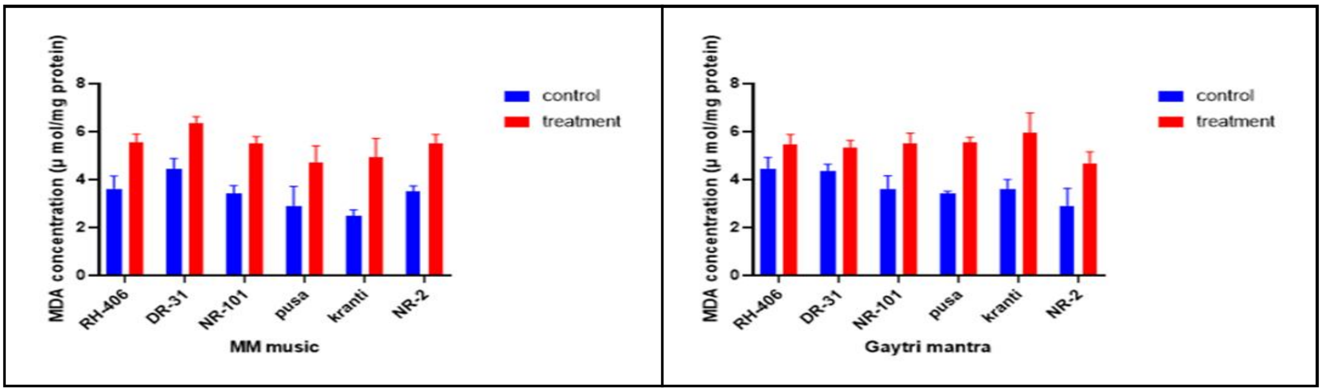

Figure 1: Effects of morning meditation music and Gaytri mantras on MDA content with all six different varieties of *B. juncea*. All experimental were exposed to sound frequency ranging from 70 dB to 90 dB Hz. Significance is denoted by symbols; the *p* values are less than 0.05.

Similarly, the effect of Gaytri mantra music treatment on the content of MDA was also increased on the 15th day. Among these varieties, the maximum content of MDA was found in Pusa, NR-101, and NR-2 as compared to DR-31, RH-406, and Kranti on 15 days. Eventually, we also observed that all six varieties of *B. juncea* had better responses and were slightly different from each other compared to the control.

3.2 Catalase

As can be seen in Figure 2, in a comparison of CAT activity between musical treatment and control in the seedlings of all six different varieties of *B. juncea* on 15 days, significant differences ($p \leq 0.05$) were observed. In the morning meditation musical treatment, analysis of the corresponding results showed that the highest catalase activity was in Kranti and the lowest catalase activity was in NR-2. Similarly, by effect of Gaytri mantra music treatment, we measured the highest catalase activity in RH-406 and the lowest catalase activity in NR-101. On the other hand, without a musical treatment plant or control plant, the maximum value was around RH-406 and the minimum value was around DR-31.

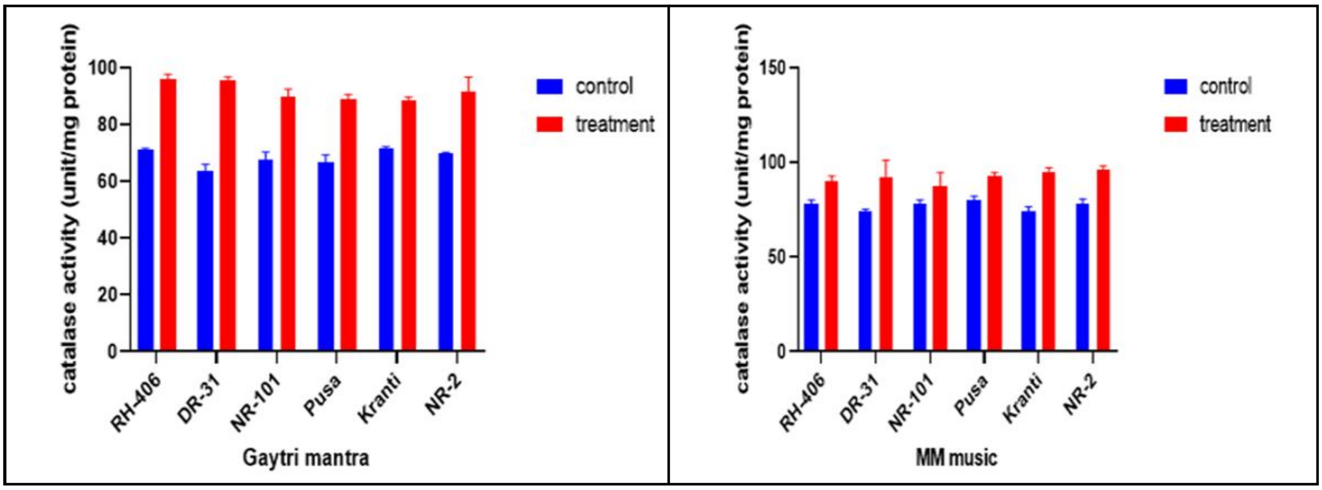

Figure 2: **Effects of morning meditation music and Gaytri mantras on catalase activity of with all six different varieties of *B. juncea*. All experimental were exposed to sound frequency ranging from 70 dB to 90 dB Hz. Significance is denoted by symbols; the *p* values are less than 0.05.**

3.3 Peroxidase (POD) (EC 1.11.1.7)

As can be seen in Figure 3, in a comparison of peroxidase activity between musical treatment and control in the seedlings of all six different varieties of *B. juncea* on 15 days, significant differences ($p \leq 0.05$) were observed. In the morning meditation musical treatment, analysis of the corresponding results showed that the common peroxidase activity was in RH-406, Pusa, and NR-2, and the lowest but most common peroxidase activity was in DR-31 and NR-101. Similarly, by effect of Gaytri mantra music treatment, we measured the highest peroxidase activity in Kranti and the lowest peroxidase activity in RH-406. On the other hand, without a musical treatment plant or control plant, the maximum value was around DR-31, and the minimum value was around RH-406.

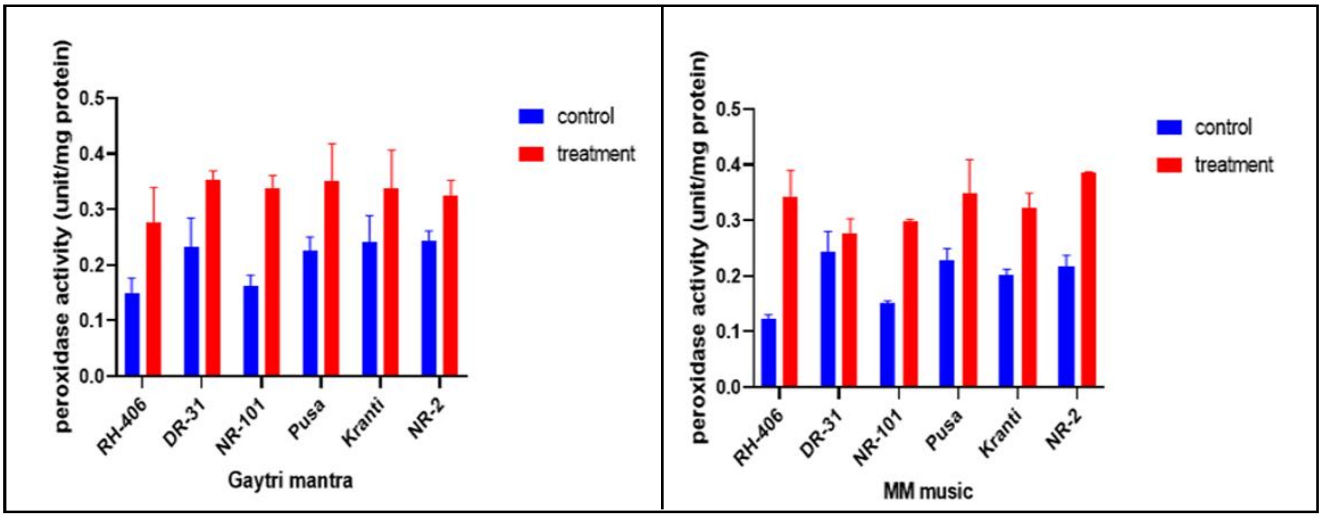

Figure 3: **Effects of morning meditation music and Gaytri mantras on peroxidase activity of with all six different varieties of *B. juncea*. All experimental were exposed to sound frequency ranging from 70 dB to 90 dB Hz. Significance is denoted by symbols; the *p* values are less than 0.05.**

3.4 Superoxide dismutase (SOD)

As can be seen in Figure 4, a comparison of SOD activity between musical treatment and control in the seedlings of all six different varieties of *B. juncea* on 15 days revealed significant differences ($p \leq 0.05$). In the morning meditation musical treatment, analysis of the corresponding results showed that NR-101 had the highest superoxide dismutase activity and Pusa had the lowest superoxide dismutase activity. Similarly, by effect of Gaytri mantra music treatment, we measured the highest superoxide dismutase activity in Pusa and the lowest superoxide dismutase activity in NR-101. On the other hand, without a musical treatment plant or control plant, the maximum value was around Kranti and the minimum value was around NR-2.

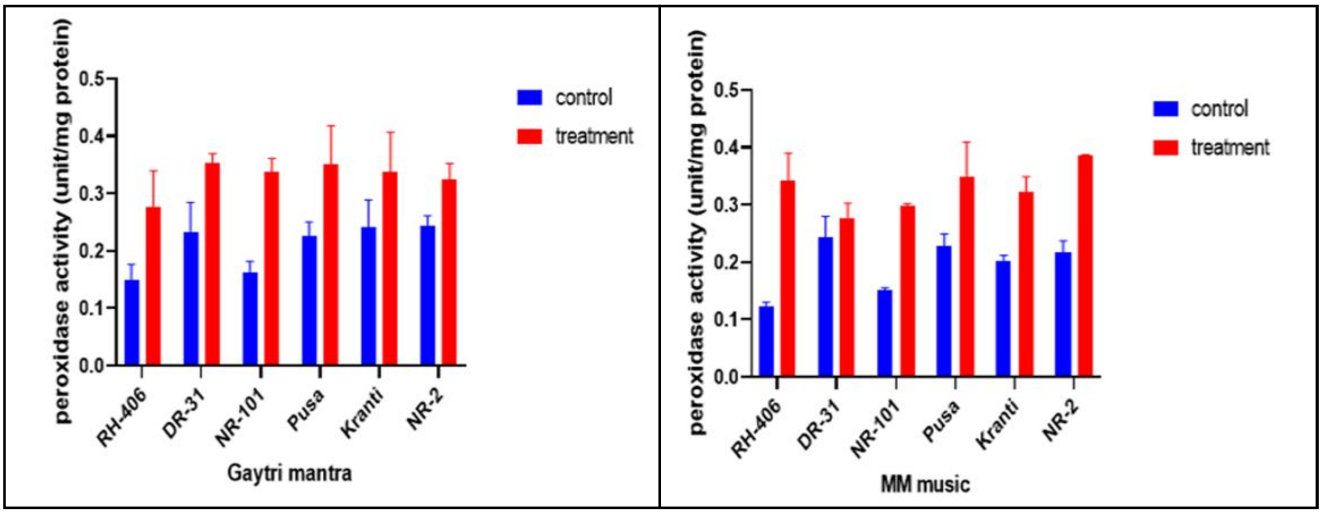

Figure 4: Effects of morning meditation music and Gaytri mantras on Superoxide dismutase activity of with all six different varieties of *B. juncea*. All experimental were exposed to sound frequency ranging from 70 dB to 90 dB Hz. Significance is denoted by symbols; the *p* values are less than 0.05.

4. Discussion

Protein kinases and ROS-scavenging enzymes have been reported to become more active in response to SVs. Superoxide dismutase (SOD), catalase (CAT), peroxide dismutase (POD), ascorbate dismutase (APX), and peroxidase are examples of protective enzymes whose activity is elevated in response to SV stimulation. This demonstrates that the level of ROS in the cell temporarily increases in response to SV activation. The first increase in malondialdehyde (MDA), a by-product of lipid peroxidation by ROS, after SV therapy provides direct evidence for ROS accumulation, which eventually declines with increasing antioxidant activity (Li *et al.,* 2008).

According to previous research, peroxidase (POD) and catalase (CAT), the two primary enzymes in plants' antioxidant systems, were found to be more active when music and sound were applied (Abedi *et al.,* 2020). Abiotic stress causes cells to accumulate H_2O_2, and in these circumstances, certain antioxidant enzymes, including CAT and POD, become more active. H_2O_2 is thought to be able to start signalling pathways. Plant cells produce free radicals such as O_2, OH, and $^1O_2$ through a variety of activities. These free radicals have a potent ability to oxidise a wide variety of useful compounds. Because H_2O_2 can produce more OH and $^1O_2$ free radicals, its removal is crucial for maintaining low levels of free radicals in plant cells (Fahimirad *et al.,* 2013). H_2O_2 is predominantly broken down by peroxidase (POD) and catalase (CAT), whereas superoxide dismutase (SOD) removes O_2. Numerous plant defensive enzymes, including SOD, POD, and CAT, were stimulated by sound waves to a greater extent.

This had a significant impact on lowering the buildup of active oxygen species (AOS), which may shield cells from oxidative damage. Additionally, distinct cell compartments may activate different defence mechanisms, obviating the need for excessive AOS production. In addition, the *Dendrobium candidum* plant is subjected to sound wave stress, which increases the content of malondialdehyde, a consequence of the breakdown of polyunsaturated fatty acid hydroperoxides (Gadjev *et al.,* 2006; Li *et al.,* 2008).

This study offers comparative data on how plant reactions to Indian classical music can differ from those without musical intervention. Gaytri mantra music and therapies, as well as MM music, both enhanced growths, turned on the ROS scavenging mechanism, and boosted secondary metabolism. Surprisingly, more of the general positive effects on seedlings of *B. juncea* were noticed. Based on the findings of this study, it may be hypothesised that plants have a diagnostic system that can distinguish the nodes of Indian classical music from a mellow melody. It appears that plants have a preference for sound. This study also emphasises the importance of paying close attention to the physical properties of sound, such as frequency and intensity, in order to understand the mechanisms at play in the interaction between plants and music. But traditional Indian music may have negative effects on plant immunity and growth, which is why further research is required.

5. Conclusion

The relationship between music and plant development has long been the subject of scholarly discussion. From previous research, we were intrigued to learn the results of these experiments and what the researchers had to say about how music affects plants. As we dug deeper into the subject, we discovered that studies on the relationship between music and plant growth in plant growth chamber settings have been done, and they appear to indicate that listening to music while effective germination occurs may really encourage plant growth. Plant growth is significantly influenced by music. Plants appreciate music, and they respond better when exposed to the right kind of music. This can be because different musical genres create distinct vibrations. So, to boost yield, musical therapy can be used in agriculture because, at the right frequency and intensity, sound stimulation encourages callus growth. Plant growth is positively influenced by sound. It has been proved by the present work that Indian classical music and Vedic mantras both promote plant growth by altering the metabolism and secondary metabolites' profile.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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