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Effect of Synchronized Sound Waves in the form of Indian Classical *Ragaas* on Phytohormonal Analysis of Medicinal Plant Species

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Abstract **Original Research Article**

Music, based on the synchronized sound waves has a tremendous impact on the living beings. Different frequencies of synchronized sound are now-a-days used in therapy (being popularly called as music therapy). However, the impact of music on physical and physiological processes of the living beings has been acknowledged since ages. Rhythmic and comforting music has an influence on behavior of all living organisms such as humans, plants and animals. According to various studies, Indian Classical music has been confirmed to encourage plant growth positively and it is observed to dominate the other important genres of music such as metal-rock, rock, rap, pop, and monotonous sounds. The present research work was aimed at finding the exclusive impact and effect of the synchronized sound waves and their frequencies in different strings and closed-pipe Indian classical instrumental music displayed through various *Ragaas*, viz: *Raga Kedar* (flute), *Raga Kedar* (santoor), *Raga-Rageshree* (sitar), *Raga-Bhairavi* (flute), *Raga- Shree* (Sarangi), *Raga- Milan ki Todi* (Sarod), *Raga- Ramkali* (Sitar) on phytohormonal analysis in Insulin (*Chamaecostus cuspidatus*) and stevia (*Stevia rebaudiana*). Enhanced phytohormonal levels were observed in the plant species treated with different Indian classical *Ragaas.* In both *Chamaecostus cuspidatus* and *Stevia rebaudiana* the total concentration of GA_3 was found to be 37.33mg/ml and 96.77mg/ml in treated plants respectively. Similarly in *Chamaecostus cuspidatus* and *Stevia rebaudiana* the concentration of IAA at 222nm was found to be 2.33 and 7.71mg/ml and at 280nm was found to be 8.45mg/ml and 41.90mg/ml in treated plants respectively.

Keywords: Music therapy, Indian Classical Ragas, synchronized sound waves, Indian classical instrumental music, phytohormonal analysis.

1. INTRODUCTION

1.1 Sound

Sound is produced by the vibratory motion of mass in contact with air. Similarly, a vibratory motion with specific frequency represents a musical sound. Notes, in music, produce sounds with specific frequencies. Music is generated only with these notes aligned in sequence with respect to time. Frequency may be gauged impartially as it is a physical entity but pitch is its non-physical or conceptual equivalent, so it cannot be measured objectively. Even though, the link between frequency and pitch is not linear; however, it is generally found that the pitch of a pure sound is increased simultaneously when its frequency is raised. A recorder creates practically a pure tone. Sound in terms of music is often persistent frequencies held for a constant period. When the sound waves reveal unbroken or continuous pitches i.e. they are musical sounds, the consequential superposition can sound moreover pleasant (consonant), or unpleasant (dissonant) (Lapp, 2002). The subsequent stage to comprehend the reason of in what way the physical systems can be prepared to vibrate in frequencies analogous to the notes of the musical scales is in following the physics of music and musical instruments. Every musical instrument shows the phenomena of resonance and standing waves.

1.2 Musical Notes and Ocaves

Musical tones are generally (almost) periodic. The fact that these sounds are periodic is the key to their having definite pitches. Sounds which are periodic have a definite pitch and musical sounds are perfectly periodic. Octave which can simply be known as a doubling in frequency (40 Hz is one octave greater than 20 Hz) is one dynamic concept related to music. The audible sound range has been arranged into several '*octaves*' (Shah, 2001). An individual octave has been classified into intervals, on the basis of some mathematical principles. The frequencies regarding Western music are compiled in a geometric series and an octave is categorized into twelve parts with the adjacent frequencies having a constant ratio of twelfth root of two. Saptak, which is equivalent to octave, in Indian Classical Music, is composed of twenty-two tones referred to as 'Shruti'. Indian Classical Music has the seven pure notes called '*shuddha swaras'*. The 'shuddha swaras' are surrounded by the microtones i.e. shrutis, which exhibit different frequency range (Table 1).In addition to these seven notes, the other supplementary five notes called the '*vikrit swaras'* are known, which are *Re Komal, Ga Komal, Ma tivra*, *Dha Komal and Ni Komal*. While '*Komal*' refers to a decrease in the frequency form the adjacent pure note, a rise in frequency means '*Tivra*'.

Table 1: Frequency ratios of 'shruti' values (Source: Shah, 2001)

Shruti	Frequency ratio ($f=v/\lambda$)	Frequency (Hertz)
Sa	1/1	240
Re1	32/31	252.8
Re2	16/15	256
Re3	10/9	266.6
Re4	9/8	270
Ga1	32/27	284.4
Ga2	6/5	288
Ga3	5/4	300
Ga4	81/64	303.7
Ma1	4/3	320
Ma2	27/20	324
Ma3	45/32	337.5
Ma4	64/45	341.3
Pa	3/2	360
Dha1	128/81	379
Dha2	8/5	384
Dha3	5/3	400
Dha4	27/16	405
Ni1	16/9	426.6
Ni2	9/5	432
Ni3	15/8	450
Ni4	31/16	465

*Sa-Shadja, Re-Rishab, Ga-Gandhar, Ma-Madhyam, Pa-Pancham, Dha-Dhaivat, Ni-Nishad.
* V is the wave speed, λ is the wavelength of the wave

The tone and scales in music are also an important element in music and the equally related terms are rhythm and speed. Several degrees of speed or tempo called as '*Laya*', ranging from '*vilambit*' (very slow) to ultra-fast ('*Drut*'). A total of 240 vibrations per second are produced by the note *Shadja* (Sa). Likewise, number of vibrations generated by other notes is: *Rishab* Re)-270, Gandhar (Ga)-300, Madhyam (Ma)-320, *Pancham* (Pa)-360, *Dhaivat* (Dha)-400 and *Nishad* (Ni)-450 (Thakkar et al. 2014). Hypothetically, the Indian Classical *Ragaas* can also be scientifically classified like the living organisms and such classification can be referred to as Genesis. According to the genesis of Indian Classical *Ragaas*, *Thaat* equals Class, the *Ragaas* are equivalent to Family and the *Notes* may be synonymous to speceis (Table 2).

Table 2: Genesis of Indian Classical *Ragaas* (Source: Bazmi *et al*., 2004)

Name of *Thaat* (Class)	Some well-known *Ragaas*of the corresponding *Thaat*(Family)	Properties of *Notes* (speceis) of that *Ragaas*	Time of playing
Bilawal	Bilawal, Bihag, Durga, Hansdhwani	All shuddh or natural notes	Almost all day
Kafi	Kafi, Pilu, Bageshri, Mian ki Malhar	Ga, Ni, Komal	Almost all the day
Asavari	Asavari, Jaunpuri, Darbari, Kanada	Ga, Dha, Ni Komal	Noon (10am-12am), Night (10pm-12am), Midnight (12am-2am)
Khamaj	Khamaj, Jhinjhoti, Desh	Ni Komal	Late evening (8pm-10pm)
Kalyan	Kalyan, Shuddh Kalyan, Aiman	No Komal, only Teevra Ma	Almost all day
Bhairav	Bhairavi, Gauri, Lalit, Jogiya, Ramkali	Re, Dha Komal	Early morning (6am-8am), Morning (8am-10am), Evening (6pm-8pm)
Bhairavi	Bhairavi, Bilaskhani, Todi, Malkaus	Re, Ga, Dha, Ni komal	Morning (8am-10am), Noon (10am-12am), Midnight (12am-2am)
Marwa	Marwa, Jait, Vibhas,	Re Komal, Ma Teevra	Almost all the day
|Poorvi	Purvi, Shree, Basant	Re, Dha Komal, Ma Teevra	Predawn (2am-4am), Dawn (4am-6am), Dusk (4pm-6pm), Evening (6pm-8pm)
Todi	Todi, Multani, Gurjari Todi	Re, Ga, Dha Komal, Ma Teevra	Morning (8am-10am), Dusk (4pm-6pm)

1.3 Music & plants

Sound waves of different variation are known to affect the growth of the plants. Synchronized sound waves of Indian classical music is also known to affect or stimulate the seed germination, flowering, fruiting and fruit ripening in several plants (Godbole 2013). Several works and observations showed that plants respond to different types of music. It can show both detrimental and stimulatory effect in its growrh and development. Sound with hard-core vibrations causes major detrimental effects on the plant growth and development (Chivkula and Ramaswamy, 2014). Plants accept Indian classical musicand showed an increased growth and development, which is well infered from our present experimental research work. We observed the effect of Indian classical music on plants at a regular basis and could conclude from our observations how aptly our experimental plants responded to the tune of Indian classical music.

2. MATERIALS AND METHODS

2.1 Materials used were as follows:-

a) Acoustic chamber
b) Music system with the main unit of frequency 50 Hz-200Hz and two satellite units (with a frequency range of 200Hz-1800Hz)
c) Selected plant materials (*Stevia rebaudiana* and *Chamaecostus cuspidatus*)

2.2 METHODS

a) Phytohormonal analysis were carried out in both the selected control and experimental plant species (Ergũn *et al.* 2002).
b) Statistical analysis were done for ± standard error and mean of each value.

3. RESULTS AND DISCUSSION

3.1 Results

The phytohormonal analysis was done in two medicinal plants, *Stevia rebaudiana* and *Chamaecostus cuspidatus* following the protocol given by Ergũn *et al.*, 2002., where the O.D. value (optical density) was measured using UV/Vis spectroscopy. Spectrophotometric analysis was done using 222nm and 280nm for IAA, and 254nm for GA_3. The experiments was repeated three times in each of the case. Total IAA for both the wavelengths that is 222nm and 280nm and total GA3 for 254 nm was calculated as the sum of the free and bound IAA and GA3. An increased O.D. value of both control and treated plant in both the selected medicinal plant species was observed as compared to standard value of phytohormones for both IAA and GA3. However, both the treated medicinal plants showed an increasing O.D. value; hence increased phytohormonal (IAA and GA3) concentration as compared to control plants. The results are given in tabular forms **(**Table 3, 4 and 5 represents *Chamaecostus cuspidatus* and table 7, 8 and 9 represents *Stevia rebaudaiana*) and graphical forms (Figure 1, 2 and 3 represents *Chamaecostus cuspidatus* and Figure 4,5 and 6 represents *Stevia rebaudiana*). The concentration of free, bound and total IAA and GA3 for *Chamaecostus cuspidatus* and *Stevia rebaudiana* is presented as ± standard error mean for each value in Table 6 and Table 10 respectively.

Table 3: IAA concentration in *Chamaecostus cuspidatus*

Treated sample	Standard	Control		Experimental	
	O.D.	O.D.	Concentration in mg/ml	O.D.	Concentrationin mg/ml
Free	2.257	0.276	2.45	0.554	4.90
Bound	2.257	0.234	2.08	0.392	3.54

*IAA concentration in mg/ml at 222nm.

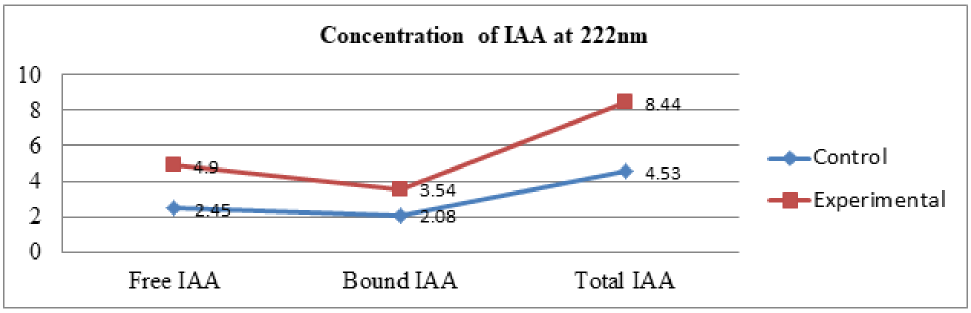

Figure 1: Graph showing IAA concentration at 222nm in *Chamaecostus cuspidatus* (free, bound and total)

Table 4: IAA concentration in *Chamaecostus cuspidatus*

Treated sample	Standard	Control		Experimental	
	O.D.	O.D.	Concentration in mg/ml	O.D.	Concentrationin mg/ml
Free	2.482	0.099	0.79	0.176	1.42
Bound	2.482	0.089	0.72	0.154	1.24

***IAA concentration in mg/ml at 280nm**

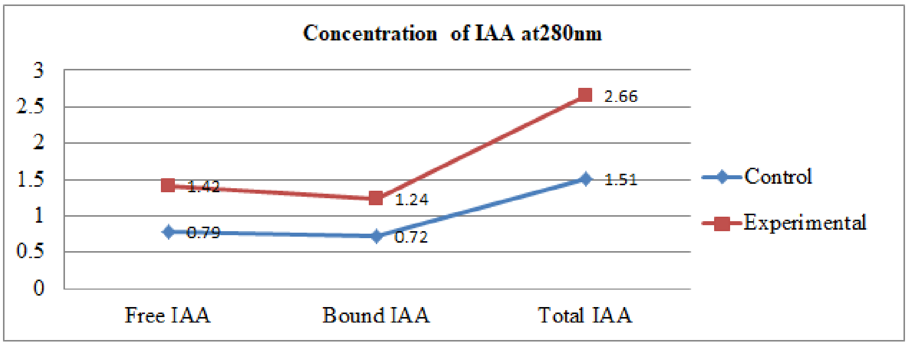

Figure 2: Graph showing IAA concentration at 222nm in *Chamaecostus cuspidatus* (free, bound and total)

Table 5: GA3 concentration in *Chamaecostus cuspidatus*

Treated sample	Standard	Control		Experimental	
	O.D.	O.D.	Concentration in mg/ml	O.D.	Concentration in mg/ml
Free	0.020	0.009	11.66	0.011	9.66
Bound	0.020	0.017	19.66	0.019	17.66

*GA3 concentration in mg/ml at 254nm.

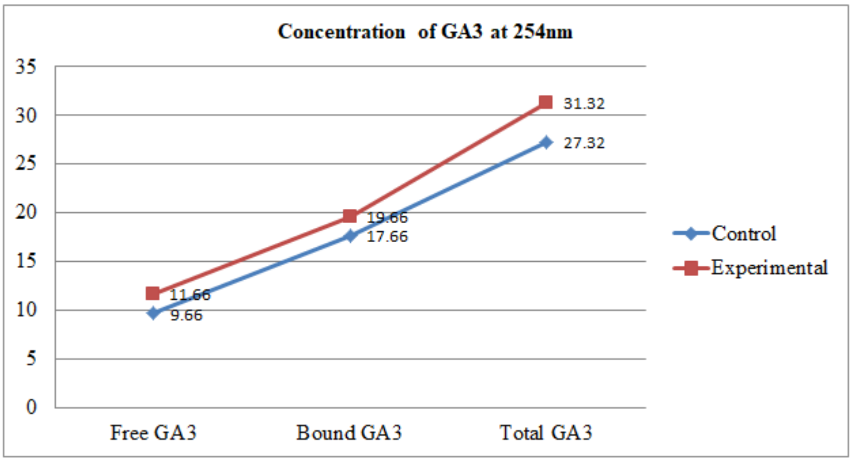

Figure 3: Graph showing GA3 concentration at 254nm in *Chamaecostus cuspidatus* (free, bound and total)

Table 6: The concentration of free, bound and total IAA and GA3 equivalent assayed from *Chamaecostus cuspidatus* represented as ± standard error mean for each value

Hormones	Form of hormones	Control	Experimental
	Free IAA	2.45±0.03	4.90±0.01
IAA(222nm)	Bound IAA	2.08±0.008	3.54±0.02
	Total IAA	4.51±0.05	8.45±0.01
	Free IAA	0.79±0.01	1.42±0.01
IAA(280nm)	Bound IAA	0.72±0.005	1.24±0.01
	Total IAA	1.52±0.01	2.33±0.34
	Free GA3	11.66±0.33	19.66±0.33
GA3(254nm)	Bound GA3	9.66±0.33	17.66±0.33
	Total GA3	21.33±0.66	37.33±0.66

Table 7: IAA concentration in *Stevia rebaudiana*

Treated sample	Sandard	Control		Experimental	
	O.D.	O.D.	Concentration in mg/ml	O.D.	Concentration in mg/ml
Free	2.257	2.412	21.38	2.491	19.51
Bound	2.257	2.201	19.51	2.234	19.80

*IAA concentration in mg/ml at 222nm

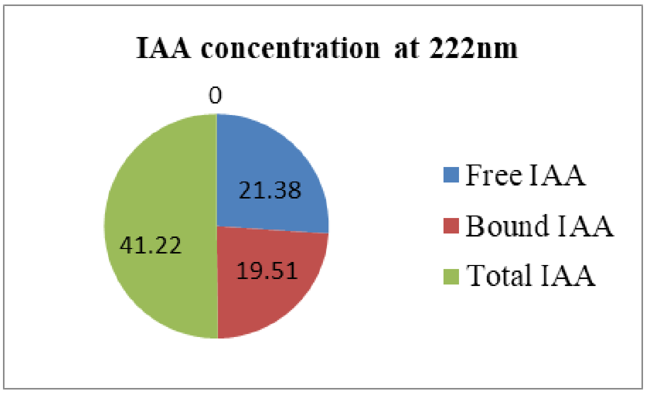

Figure 4: Graph showing IAA concentration at 222nm in *Stevia rebaudiana* (free, bound and total)

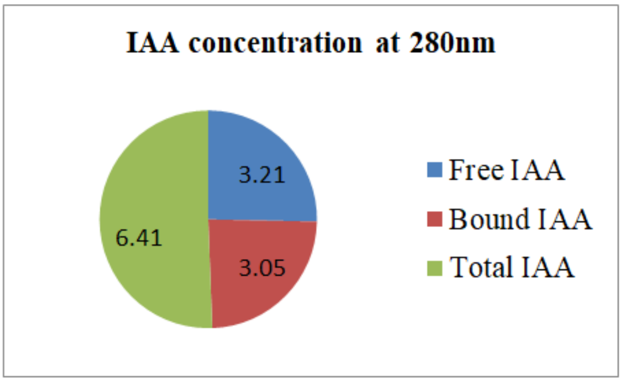

Figure 5: Graph showing IAA concentration at 280 nm in *Stevia rebaudiana* (free, bound and total)

Table 8: IAA concentration in *Stevia rebaudiana*

Treated sample	Standard	Control		Experimental	
	O.D.	O.D.	Concentration in mg/ml	O.D.	Concentration in mg/ml
Free	2.482	0.399	3.21	0.526	4.27
Bound	2.482	0.378	3.05	0.426	3.43

*IAA concentration in mg/ml at 280nm

Table 9: GA3 concentration in *Stevia rebaudiana*

Treated sample	Standard	Control		Experimental	
	O.D.	O.D.	Concentration in mg/ml	O.D.	Concentration in mg/ml
Free	0.020	0.046	46	0.051	51
Bound	0.020	0.041	41.33	0.045	45.66

*GA3 concentration in mg/ml at 254nm

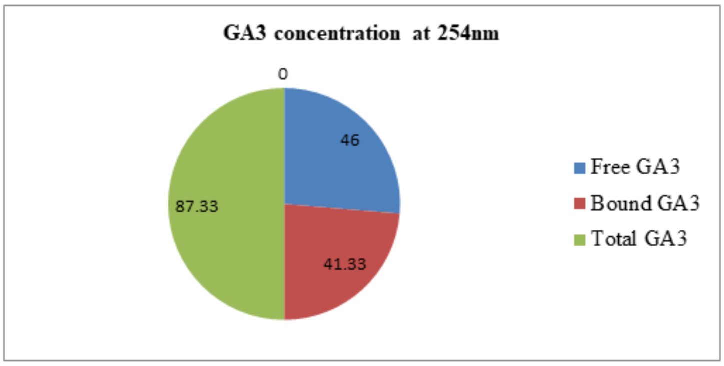

Figure 6: Graph showing GA3 concentration at 254nm in *Stevia rebaudiana* (free, bound and total)

Table 11: The concentration of free, bound and total IAA and GA_3 equivalent assayed from *Stevia rebaudiana* represented as ± standard error mean for each value

Hormones	The form of hormones	Control	Experimental
	Free IAA	21.45±0.33	22.07±0.04
IAA(222nm)	Bound IAA	19.51±0.01	19.80±0.01
	Total IAA	41.22±0.01	41.90±0.005
	Free IAA	3.21±0.012	4.27±0.02
IAA(280nm)	Bound IAA	3.05±0.02	3.43±0.01
	Total IAA	6.41±0.01	7.71±0.005
	Free GA3	46±0.6	51±0.6
GA3(254nm)	Bound GA3	41.33±0.7	45.66±1.2
	Total GA3	87.33±1.2	96.66±1.8

3.2 DISCUSSION

Plants do absorb and resonate to external frequencies in the form of sound waves as conceptualised and established by Sir Jagdish chandra Bose a Nobel Prize winner in 1977, who was renowned for his work on the physiology of plants. He believed that plants could feel and were aware of their surroundings. He was the first person to prove that plants feel pain and understand affection. He demonstrated the electrical nature of plant stimuli (like wounds, chemical agents, etc.). He researched the seasonal effect on plants and also the effect of chemical inhibitors and temperature on plants. Thus, he contributed significantly to agriculture also.

Sound waves are received by the plants and results in the faster protoplasmic movement (Godbole 2013). Due to the sound waves mechanical vibrations are generated by the charged cell membranes and wall. The mechanochemical enzymes which use chemical energy in the form of ATP to produce mechanical vibrations in cells contributes to the sound wave generation in plant cells. These mechanisms leads to the nanomechanical oscillations of cytoskeletal components of the plant cells that generates both high and low wave frequency vibrations. The synchronized sound waves in the form of Indian classical *ragaas* acts as potent plant growth stimulant and as plant protectant. Plant growth stimulant in the sense that it elicits various effects in the plants. Starting from the enhancement of seed germination in the sense showing early seed germination and healthy seedling growth. Musical sound promotes the plant growth by regulating synthesis of phytohormones Indole3-acetic acid and Gibberellic acid (Bochu *et al*., 2004; Ghosh *et al*., 2016). Plant growth promotion can also be related to that of the enhancement of photosynthetic activity. The sound waves manipulates the expression of photosynthetic related genes that leads to an increase in the photosynthetic rates in plants (Jeong *et al*., 2008).

In the present research experiments, it was also seen that there was an increment in the overall growth of the plant body. Both the medicinal plant species *Stevia rebaudiana* and *Chamaecostus cuspidatus*was subjected to soft harmonic sound waves of octaves (synchronized sound waves) through Indian musical instruments). By analysing the growth, it was noticed that there was an increment in the growth of the treated plants. The present study also showed that there was efficient increment in the phytohormonal contents of treated plants as compared to that of the untreated control plants. In both *Chamaecostus cuspidatus* and *Stevia rebaudiana* the total concentration of GA_3 was found to be 37.33mg/ml and 96.77mg/ml in treated plants respectively. Similarly the concentration of IAA at 222nm was found to be 2.33 and 7.71mg/ml and at 280nm was found to be 8.45mg/ml and 41.90mg/ml in treated *Chamaecostus cuspidatus* and *Stevia rebaudiana* respectively.

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