Impact of harmonic Octave Consonants (Classical Musical Notes) on the discrete physiognomic characters and different biochemical aspects insweetleaf viz., Stevia rebaudiana (Bertoni)

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Abstract

Music is an integral part of our nature and society (Lapp, 2002). The harmonic octave consonants and their frequencies are now-a-days used as a therapy, being popularly called as music therapy. However, the impact of music on our physical and physiological processes of living beings has been acknowledged since ages. Rhythmic and comforting music has an influence on physical and physiological conditions and behavior of living organisms such as humans, plants and animals (Ekici et al. 2007). According to various studies, Indian Classical music has been confirmed to encourage plant growth positively and it has also been observed to dominate the other important genres of music such as western classical, rockand monotonous sounds (Rettalack, 1973). The present research work is aimed at finding the exclusive impact and effect of the harmonic octave consonants 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 the distinct physiognomiccharacters and different biochemical aspects in sweetleaf viz., Stevia rebaudiana (Bertoni). The results were surprising as the treated plants showed higher efficacy in terms of both its physiognomic and biochemical aspects as compared to the control plants. The results were depicted here through tabulations and figures. Physiognomic parameters like plant height, nnmber of leaves per plant, leaf lamina length, leaf lamina breadth, leaf texture, leaf color, spread of plant in east-west and in north-south directions and diameter of the stem for a period of 30 days. Treated plants showed an increment in the growth as compared to the control and survived longer (20 days longer) than the control plants. For instance, in sweetleaf, the average no. of leaves in control was 53 per plant and 64 in treated plants. Similarly, the average height of the treated (experimental) plant was found to be greater i.e., 28 cm as compared to the lower average height of 21 cm as shown by the control (untreated) plant samples.The biochemical analysis also revealed some promising observations. On performing the biochemical experiments with the leaves of sweetleaf (both control and experimental), the total protein, carbohydrate and chlorophyll contents in the treated leaves was found to be much higher i.e., 142.25 mg/gm, 114 mg/gm and 34.69 µg/gm fresh weight, respectively. The control plants showed much lesser values such as 87.5 mg/gm, 79 mg/gm and 16.44 µg/gm for total protein, total carbohydrate and total chlorophyll content respectively. The leaves of treated (experimental) sweetleafshowed anincreased value of 75.66 mg/100gm whereas the control leaves showed 42.52 mg/100 gm of ascorbic acid.

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Abbreviations: Hz-Hertz, I-Sound Intensity, L-Sound Intensity Level, Db- Decibel

1. Introduction

Sound is a form of energy. It is a compressional wave triggered by vibrations (Wood, 1976). The vibrations that are felt on touching the surface of a speaker are equal to the vibrations disturbing the air. Sound is similar to countless other things which are invisible in our universe. It cannot be seen as well but sound can be heard as it reaches our ears in the form of waves produced when particles in the medium vibrates (Rigden, 1977). Hence, vibration in a body generates sound.

Music and noise are two different things; the former has a soothing effect and the latter is infuriating. Music is related to rhythmic and periodic vibrations while noise has no periodic vibrations (Lapp, 2002). Music for about a thousand of years have been a source of harmony for people. It is a soothing and tranquilizing form that is produced from musical instrument. Music is a fine art of sound and is basically prearranged by people to express their feelings a certain way.

Music certainly has many dimensions in stirring various physical, psychological, spiritual and social stages of awareness (Kneafsey, 1997). Currently, music is categorized as both positive and negative. Music that has valuable potentials and inspires at emotional and spiritual levels and causes relaxing, calming and healing effects is known as positive music whereas negative music induces negative emotions, irritation, bitterness, sadness, animosity and terror (Ekici et al. 2007).

Octave which can simply be known as a doubling in frequency (40 Hz is one octave greater than 20 Hz) is one dynamic conception related to music. According to Shah (2001), the several 'octaves' has been arranged on the basis of audible sound range and depending on some mathematical principles, an individual octave has been classified into intervals. An octave is categorized into twelveparts , which is the width of a semitone, i.e. the frequency ratio of the interval between two adjacent notes, is the twelfth root of two and is mathematically expressed as (Kuttner, 1975):

$^{12}\sqrt{2} = 2^{1/2} \approx 1.059463$, which is again equivalent to

$e^{1/2 \ln 2} \approx 1.059463$

This interval is divided into 100 cents. An equivalent of octave in Indian Classical Music is *Saptak* that is a constituent of twenty-two tones stated to as '*Shruti*'. The seven pure notes in Indian Classical Music are called '*shuddha swara*s' and are composed by the microtones i.e *shrutis* showing distinct frequency range (Table 1).

After the first concept of perception of sound waves by plants as suggested by Sir Jagdish Chandra Bose, a Nobel Prize winner in 1977, who was renowned for his work on the physiology of plants; Dorothy Retallack also carried out one of the first experiments in 1973 to study the relationship between plants and music. Various styles of music were used by Retallack in her experiments and she discovered that the plants showed a tendency to move away from Led Zeppelin and Jimi Hendrix but Bach organ music and jazz attracted them. Nevertheless, she found that North Indian classical music played on the sitar was their favorite but country music was seen to have no such relevance in plant life (Rettalack, 1973).

All living beings, be it animals or plants, all respond to external stimuli. Plants, the multicellular organisms also respond to various types of external stimuli including Sound vibration. These vibrations stimulate different phytochemical and biochemical reactions which in turn helps in the developmental processes of the plants. In case of human beings, sound waves set up vibration in our ear drum which in turn is perceived by the brain and help us to recognize the type of sound of varying frequencies and amplitude.

Plants, in a similar way receive vibrations through protoplast. Various studies related to the effects of sound waves subjecting to seeds and plants, generally known as sonication, has been published in scientific literature (Suslick, 1989; Joersbo and Brunstedt, 1992).The effect of music on 30 Rose (*Rosa chinensis)* plants taken in separate pots was studied (Chivukula and Ramaswamy, 2014). The plants exposed to Indian classical music exhibited the highest internode elongation, which evidently displayed that exposing the plants to *Vedic chants*and Indian classical music stimulates the growth of plants as compared to the control group and the plants those were subjected to Western popand Rock music.

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).

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

Shruti	Frequency ratio (f = v/λ)	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* and *Ni - Nishad (Ni)*,f- frequency ratio, v- speed, λ-lambda

2. Materials and methods

2.1 Materials

2.1.1 Acoustic chamber

An acoustic chamber was used with a sound system to produce the sound of known frequency through Indian Classical Instrumental Music both String music and Closed pipe music) for the experimental plants.

2.1.2 Plant material

The plant commonly called as sweetleaf (Marcinek and Krejpcio,2015) with the scientific nameStevia rebaudiana

(Bertoni) was purchased from medicinal garden at Patrapada, Bhubaneswar in large number and used as for the experiments.

2.1.3 Measuring tape andthread

2.1.3.1 Ragaas

The application of the Indian Classical Instrumental music notes (Octaves) through different *Ragaas* were applied to our experimental plant set in the following time sequence: -

1. Raga Kedar- Flute (closed pipe), by Pandit Pannalal Ghosh (38:14) during early morning between 6am-8am, followed by-
2. Raga Kedar- Santoor (stringed instrument), by Pandit Shiv Kumar Sharma (23:14) in the Morning time between 10am-12pm,
3. Raga Rageshree- Sitar (stringed instrument), by Nikhil Banerjee (29:15) in the evening between 4pm-6pm followed by-
4. Raga Bhairavi- Flute (closed pipe), by Pandit Hari Prasad Chaurasia (9:50)

All the above four mentioned *Ragaas* have been observed to promote seed germination and vegetative growth. Physiognomic studies were conducted on an interval of four days for a month. A thread was used to measure the height of the plant, leaf lamina length, leaf laminabreadth, spread of the plant in east-west and north-south directions. It was then placed over a 60 cm long measuring tape and the measurement data were recorded in table.

3. Methodology

The plants were treated with the above harmonic sound frequencies of musical notes for 4 hours at frequent intervals daily.

3.1 Physiognomic study

All the plants were kept inside the acoustic chamber. The control plants were taken outside of the chamber when the treatment was given. Different aspects of physiognomic studies like height of the plant, leaf lamina length per plant, leaf lamina breadth per plant, spread of the plant in east- west direction and in north- south direction, and diameter of the stem were considered. These aspects were measured in every four days.

3.2 Biochemical Analysis of Leaves

To study the effect of these Indian Classical instrumental Music on biochemical aspects through various observations and inferences from the experiments, a series of biochemical tests were performed in order to estimate the quantity of total proteins, total carbohydratesand total chlorophyll contents in the leaves of *Stevia*, treated with*ragaas* versescontrol.

3.3 Protein estimation

The amount of protein was quantified by using the method of Lowry *et al* 1951 (Tambe *et al*. 2011). 500mg per 100gm fresh weightof both control and treated leaf samplewas taken for analysis.

3.4 Carbohydrate estimation

Anthrone method or Hedge method, 1962 (Das *et al*. 2010) was used for carbohydrate estimation. 100mg per 100gm fresh weight of both control and treated leaf sample was taken for analysis.

3.5 Chlorophyll estimation

Chlorophyll is extracted in 80% acetone and the absorbance are read at 663 and 645 nm in a spectrophotometer, using the absorption co-efficient, the amount of chlorophyll is calculated (Arnon,1949). 500mg per 100gm fresh weight of leaf sample was taken for analysis.

Formulas

The amount of chlorophyll present was calculated by the following formulae,

Mg chl a /gm fresh weight = 12.7(A663) – 2.69 (A645)*V/1000*W

Mg chl b / gm fresh weight =22.9(A645) 4.68(A663)*V/1000*W

Mgtotalchl/gm fresh weight = 20.2(A645) +8.02(A663)*V/1000*W

3.6 Estimation of Ascorbic acid

Ascorbic acid is estimated by DCPIP method (Harris and Ray, 1935) inboth control and treated leaf sample. 500mg per 100gm fresh weight of both control and treated leaf sample of sweetleaf was taken for analysis.

Ascorbic acid content (mg/100g) =0.5*V_2/V_1*total volume of stock solution/vol. taken for analysis*100g/wt. of sample taken (g), Where, V_1= volume of dye consumed for standard (ml), V_2=volume of dye consumed for sample (ml)

4. Results

Several studies have proved that there is an association between sound vibrations and plants lives and that both are profoundly interconnected to each other (Rettalack, 1973). While sound with a low amplitude improves plant growth, likewise, any type of sound vibrations with large amplitude values for e.g., rock music declines plant growth (Rettalack, 1973).

The present research work embraces two important aspects of the plant growth and the physiological processes involved therein were taken into consideration:

I. Effect of harmonic octave consonants on the physiognomy of sweetleaf,*Stevia rebaudiana*(Bertoni).
II. Effect of harmonic octave consonants on the biochemical analysis of sweetleaf, *Steviarebaudiana*(Bertoni).

4.1 Effect on physiognomy of plants

Physiognomy is amalgam of external features and different growth forms in plants. It is one of the most pivotal studies in case of plant classification.

4.2 Physiognomic study of Stevia rebaudiana(Bertoni)

To perform the experiment two sets were taken; one set with 4 plants as control and the other set with 4 plants as treated. Physiognomic parameters like plant height, no. of leaves per plant, leaf lamina length, leaf lamina breadth, leaf texture, leaf color, spread of plant in east-west and in north-south directions and diameter of the stem for a period of 30 days.

Treated plants showed an increment in the growth as compared to the control and survived longer (20 days longer) than the control plants. The average no.of leaves in control was 53 per plant and 64 in treated plants and the rest all data are given in tabular form. The observations are shown in tables and figures (Table2;Figure 1; Figure 2;Figure 3;Figure 4).

Physiognomic parameters	Control (average) (cm)	Treated (average)(cm)
Ph	21.5	28
Sop-(E-W)	22.67	25.71
Sop-(N-S)	21.5	28
Lll	2.34	3.5
Llb	1.2	1.3
Dos	1.8	1.9

Table 2. Measurements of physiognomic parameters in sweetleaf (Stevia rebaudiana)after 30 days of treatment with music

Ph-Plant height,Sop- (E-W)- Spread of plant (East-West), Sop-(N-S)- Spread of plant (North-South), Lll- Leaf lamina length, Llb- Leaf lamina breadth, Dos- Diameter of Stem

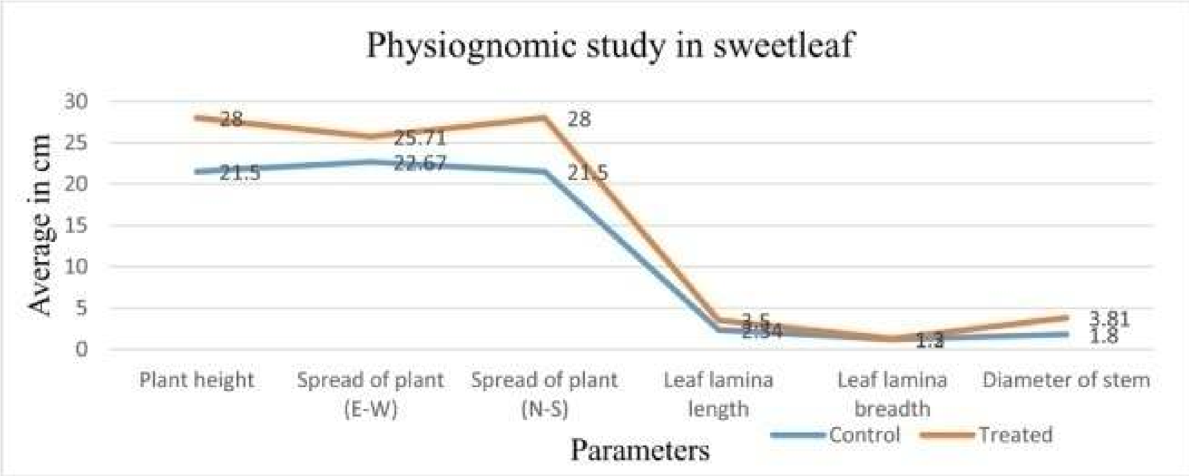

Figure1. Measurements of physiognomic parameters in sweetleaf (Stevia rebaudiana)

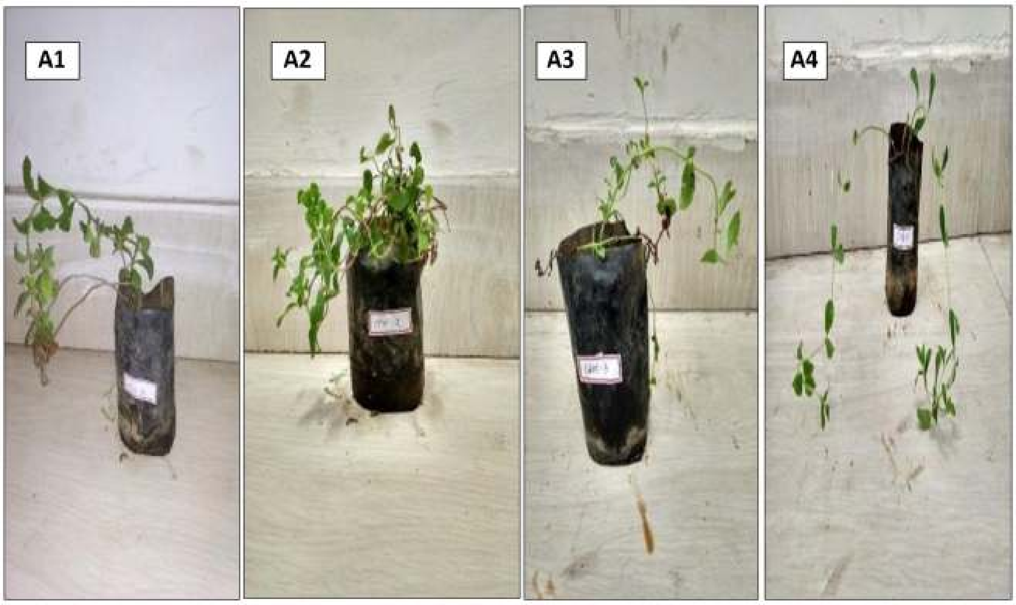

Figure 2.Sweetleaf (Stevia rebaudiana) plants before treatment (A1-A4)

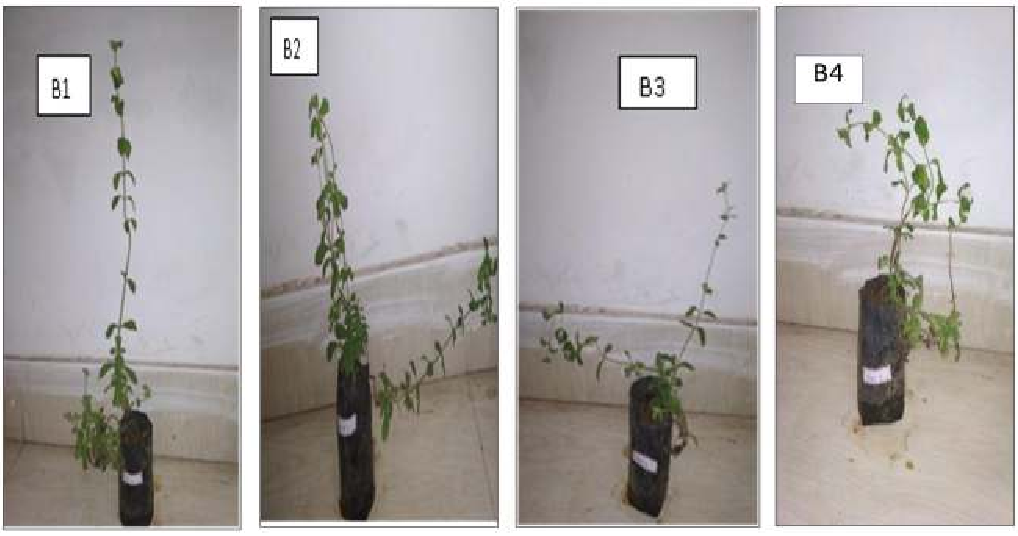

Figure 3. Treated sweetleaf(Stevia rebaudiana) set up after the period of 30 days (B1-B4)

Figure 4. Control sweetleaf (Stevia rebaudiana) plants after 30 days (C1-C4)

4.3 Effects of harmonic octave consonants onthe biochemical aspects of sweetleaf (Steviarebaudiana)

Protein estimation

Effect of harmonic octave consonants on total protein contents

Leaves of both control and treated were taken for extract preparation. The total protein content of the leaf samples was estimated by the Lowry et al 1951 (Tambe et al. 2011) method. The data generated is recorded in table (Table 3) and analyzed in figure (Figure 5).

Table 3. Total protein content of control and treated plants of sweetleaf (Stevia rebaudiana)

Treatment	Total protein content (500mg/100gm fresh weight)
Control	87.5
Treated	142.25

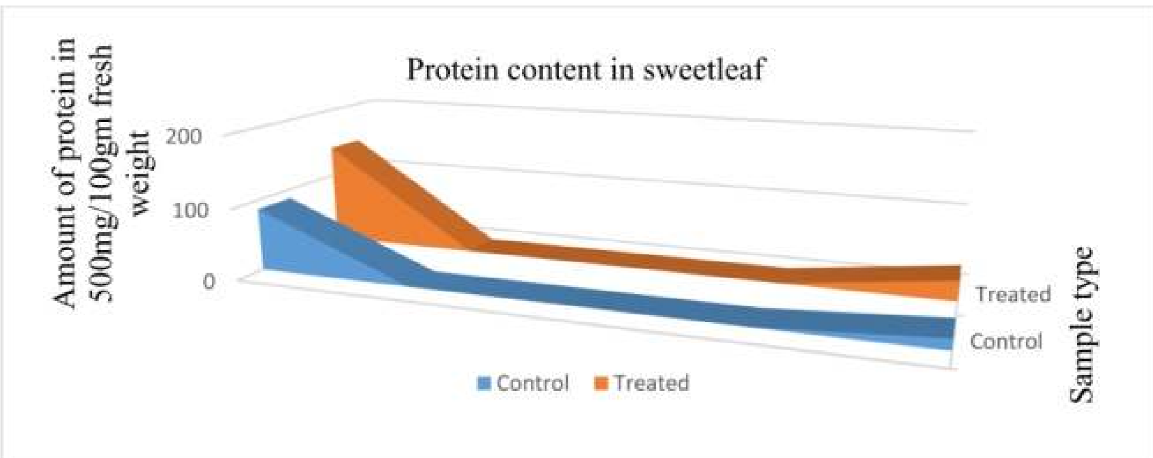

Figure 5. Total protein content of controland treated plants of sweetleaf (Stevia rebaudiana)

4.4 Total carbohydrate estimation

Effect of harmonic octave consonants on total carbohydrate content

Young leaves were selected from the treated plants. The extract was prepared from leaves of both control and treated plants and the total carbohydrate was estimated using the Anthrone method or Hedge method, 1962 (Das et al. 2010). Equal amount of leaf sample was taken for both control and experiment. The carbohydrate concentration was found higher in the treated plants as compared to the control plants. The data are recorded in table (Table 4) and analyzed in figure (Figure 6).

Table 4. Total carbohydrate content of control and treated plants of sweetleaf (Stevia rebaudiana)

Treatment	Total carbohydrate content (100mg/100gm fresh weight)
Control	79
Treated	114

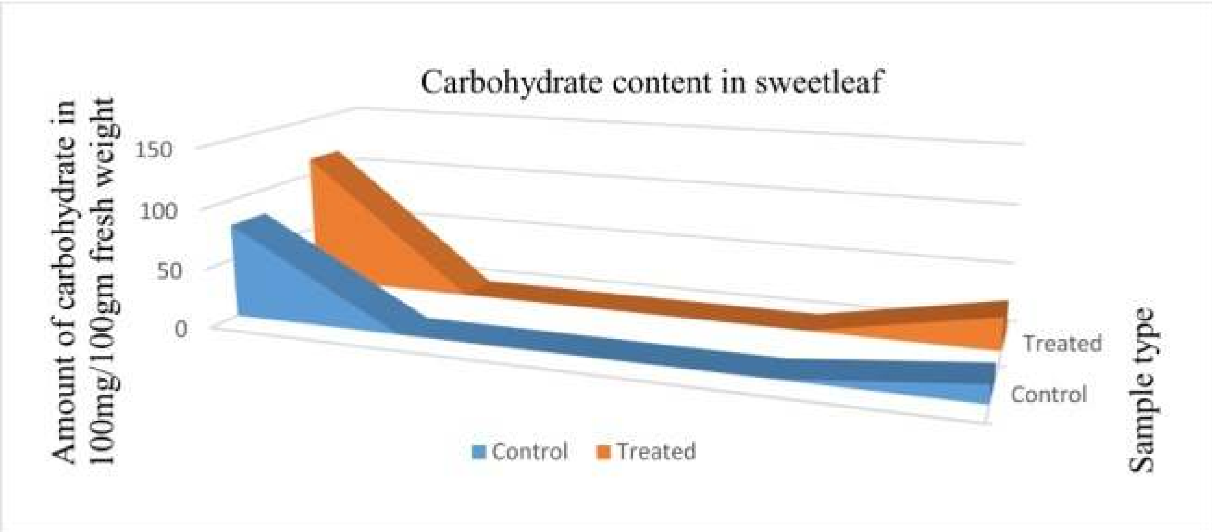

Figure 6. Total carbohydrate content of control and treatedplantsof Stevia rebaudiana

4.5 Chlorophyll estimation

Effect of harmonic octave consonants on chlorophyll content

The control and treated plants were subjected to chlorophyll estimation. The acetone method was used for estimation of chlorophyll content. For calculations of chlorophyll a, chlorophyll b and total chlorophyll content Arnon's (1949) formulae were used. The chlorophyll a chlorophyll b and total chlorophyll content were found effectively higher in treated plants than in control plants. The data are recorded in table (Table 5) and analyzed through figure (Figure 7).

Table 5. Chlorophyll a, Chlorophyll b, and total chlorophyll content of sweetleaf (Stevia rebaudiana)

Treatment	Chlorophyll a (500µg/ 100gm fresh weight)	Chlorophyll b (500µg/ 100gm fresh weight)	Total chlorophyll content (500µg/ 100gm fresh weight)
Control	17.75	19.70	16.44
Treated	24.86	38.78	34.69

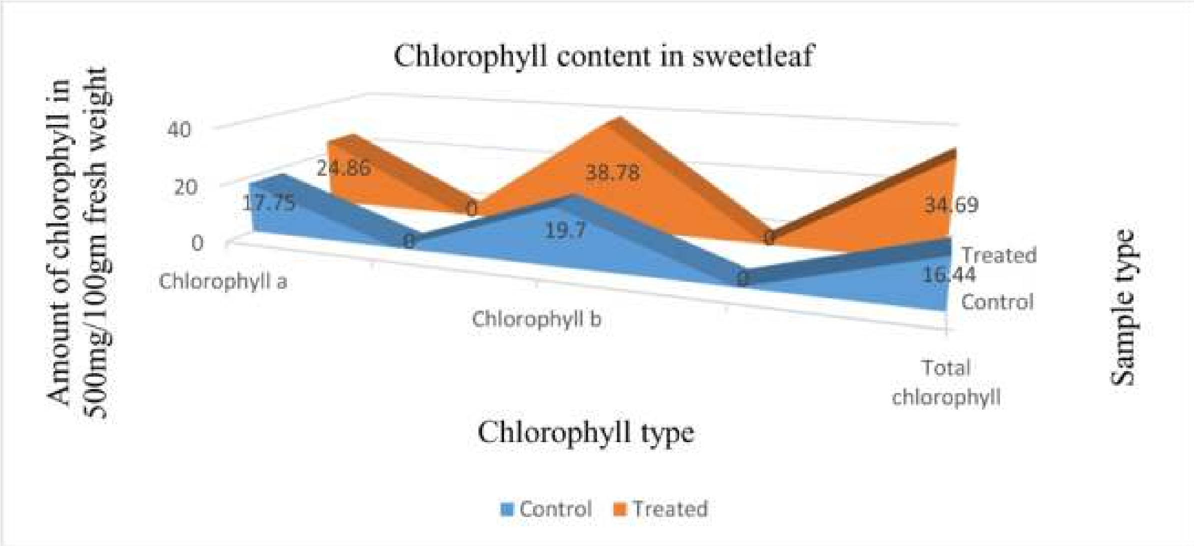

Figure 7. Chlorophyll a, Chlorophyll b, and total chlorophyll content ofsweetleaf (Stevia rebaudiana)

4.6 Estimation of ascorbic acid inStevia rebaudianaby DCPIP method

Ascorbic acid, also known as vitamin C is necessary for wound healing and many other functions in the body. Sweetleaf also shows some amount of ascorbic acid i.e., 14.98 mg·100 g-1 (Bugaj et al., 2013).Because of its immense importance and also due to its high presence, this analysis was carried out in the plant, *Stevia rebaudiana*. The analysis was carried out in the leaf of *Stevia rebaudiana.* Leaf of the treated plant showed an elevated quantity of the ascorbic acid as compared to that in the control plants. The data has been presented in the table (Table 6) and figure (Figure 8) below.

Table 6. Ascorbic acid content (500mg /100gm) in sweetleaf(Stevia rebaudiana)

Plant parts	Control	Treated
Leaf	42.52	75.66

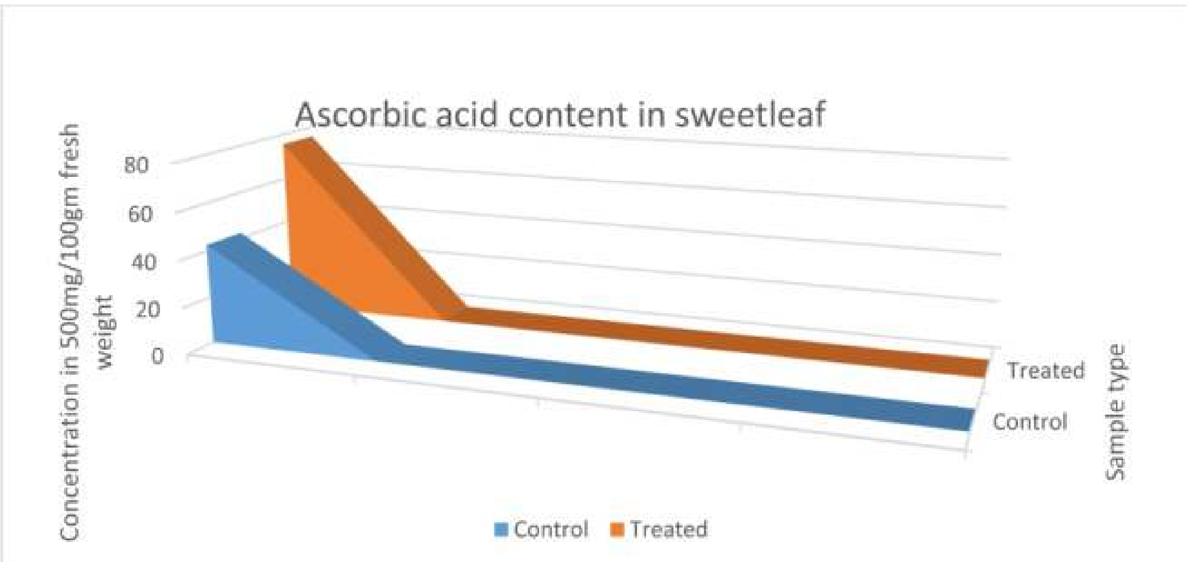

Figure 8: Ascorbic acid content (500mg /100gm) incontrol and treated leaf sample of sweetleaf (Stevia rebaudiana).

5. Discussion

Audible sound (20–20000Hz) widely exists in natural world. However, the interaction between audible sound and the growth of plants is usually scanty in biophysics research. Not much effort has been put forth in studying the relation of plant processes and audible sound.

A certain study has revealed that plants have mechano-sensitive channels that perceive sound vibrations. In another experiment it was shown that sound vibrations can alter tensions in biological membranes that could possibly stimulate signaling pathways through the activation of the mechano-sensitive channels in the membranes of plant cell (Haswell and Meyerowitz, 2006). Other experiments carried out showed that certain frequencies of sound vibrations can induce seed germination, root elongation, callus growth, and cell cycling (Gagliano, 2013). An experiment was conducted in which 12 tobacco plants were exposed to noise and showed overall 40.6 % decrease as compared to the control plants (Woodlief et al.,1969).

In the present research study, it was also seen that there was an increment in the overall growth of the plant body compared to the control though not much of difference was found. Stevia rebaudiana (Bertoni) when subjected to soft harmonic sound vibrations, the results showed a difference i.e., there was an increment in the physiognomic parameters of the treated plant as compared to the control (untreated) plant. The possible explanation to this would the perception sound vibration and relay of extended signaling pathways leading to the overall increased growth of the plant and leaves.

Audible harmonic frequencies not only accelerate growth but also significantly influences the concentration of various metabolites; e.g., chlorophyll and starch are increased by it (Sharma et al. 2015). Also, musical vibration stimulates water molecules within biological systems (Creath and Schwartz, 2002). As a result, the temperature, which is a measure of motion of molecules is raised due to vibration of the water molecules. The rate of metabolism is thereby, increased with the increase in temperature. Resonances from the audible harmonic sound vibration can combine directly into biological systems because they are constituted mostly of water.

Indole Acetic Acid (IAA) is an essential plant hormone that helps in plant's growth and development. Zhu and co-workers observed that IAA content in plants were found at an increased level in six species of vegetable plants when exposed to musical acoustic frequencies in comparison to the control plants (Jun-ru et al. 2011). The present study also revealed an increase in carbohydrates, proteins and chlorophyll content. The increased metabolic rate might be a reason for the observed results.

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