



Studies on the Phytochemical and Physiological Aspect on Selected Edible Plant Varieties in *Vigna radiata* L

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ABSTRACT:

The major legumes in Asia are chickpea (*Cicer arietinum* L), pigeon pea (*Cajanus cajan* L) and Mung bean (*Vigna radiata*). Mung bean is a crop plant, requiring 90–120 days of frost free conditions from planting to maturity. Adequate rainfall is required from flowering to ensure good yield. Mung bean (*Vigna radiata*) is one of the important grain legumes in the rain fed farming system in dry and intermediate zones. Mung bean enriches the soil and breaks the soil fatigue caused by cereal–cereal rotations. Including Mung bean in rice rotation, has increased the yield of paddy and the income of farmers. Legumes have long been recognized to be either sensitive or moderately tolerant to salinity. Plants grown in the salt-affected soil undergo alterations in their physiology and metabolism at a cellular as well as whole-plant level. Salinity is deleterious to plant growth from the germination stage to the flowering and fruiting stage, resulting in decreased yield. In the present study, an attempt has been made to screen the fertilizers (organic, inorganic) and salt stress against three different varieties of *Vigna radiata*. The results revealed that the effect of salt concentration is expressed in terms of less growth and development that was analysed by chlorophyll estimation, proline analysis and FTIR analysis

Keywords: *Vigna radiata*, fertilizers, chlorophyll estimation, proline analysis, FTIR analysis

1. INTRODUCTION

Mung bean (*Vigna radiata*) is one of the major rainy (kharif) season pulse crops of India. The yield and nutritional quality of Mung bean is greatly influenced by application of nutrient element along with organic manures and inoculation of seed with the phosphorus fertilizer. The crop responds favourably to application of phosphorus fertilizer¹. The rate of phosphorus fertilizer increasing crop yield, nutrient availability and uptake has been demonstrated under some soil of India but such information on these aspects in Mung bean layout been investigated under Rajasthan. Like other legume crops, Mung bean has potential to fix atmospheric nitrogen through its root nodules, which requires phosphorus for their proper growth and development. During

summer, it can also be used as a green manure crop. Being a leguminous crop, it has the capacity to fix atmospheric nitrogen. Its green plants are used as fodder after removing the mature pods.

Mung bean is an excellent source of protein (25%) with high quantity of lysine (4,600 mg/N) and tryptophan (60 mg/N) and consumed as whole grain and dal in variety of ways for table purposes. It has carbohydrate (51 %), protein (24-26 %), mineral (4 %), vitamins (3 %) and fat (1 %) ². It has bright green edible legume seeds which ripens uniformly and can be eaten raw or cooked. Mung bean seeds are an invaluable source of digestible protein for vegetarians³. In India, it is produced in Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra, Orissa, Rajasthan, Uttar Pradesh, Gujarat, Madhya Pradesh, West Bengal and Punjab constituting 11.48 % of all pulses⁴. The present study was aimed to investigate the phytochemical and physiological aspect on selected edible plant varieties on *Vigna radiata L*

2. MATERIALS AND METHODS

2.1. Mung beans (*Vigna radiata*)

The seeds of three popular Mung bean varieties Co-6, CO (Gg)-912 and traditional seeds were procured from the seed shops in Madurai district and used as plant material for this study. Different fertilizers (organic, inorganic) and salt (NaHCO₃) are used for different varieties of mung bean (Co-6, CO(Gg)-912 and traditional seed). Different concentrations such as 100 ppm and 250 ppm are used for this study. They are prepared and dissolving in water, finally used for irrigation to impose various effects. Organic and inorganic fertilizer (Sodium bicarbonate) treated seeds are sown in individual pots that contain control (100ppm, 250 ppm). Salt treated seeds of three Mung bean varieties were sown in earthen pots containing soil. The pots were labeled based on their variety and treatment.

Generally further the salt treatment (solution) was applied after the emergence and expansion of first leaves in all varieties to impart salt stress. The plants provided with equal volume of water used as control (C). The seed growth in each pots are monitored and measured at 10days interval

Place/ Location

- The healthy seeds of edible plant are collected and sowed in different pots and kept in the Green house, Govt. arts college, Melur, Madurai

Optimal temperature and Moisture for Green house

- Optimal temperature: Between 17-27 c
- Moisture: 50-70% relative humidity

2.2. PROLINE ANALYSIS

Homogenizing 0.5 gm of fresh plant material in 10 ml of 3% aqueous sulphosalicylic acid, the extract was made. The homogenate is filtered through Whatman No. 2 filter paper. 2 ml of filtrate is taken in a test tube and 2 ml of glacial acetic acid and 2 ml acid-ninhydrin are added in a sequence. The mixture is heated in the boiling water-bath for 1 h. The reaction is stopped by placing the tube in ice-bath. 4 ml toluene is added to the reaction mixture and stir well for 20-30 sec. The toluene layer is separated and warm to room temperature. The red colour intensity is measured at 520 nm. A series of standard with pure proline in a similar way is made and prepare a standard curve. Thus the amount of proline in the test sample (value) is compared with the standard proline curve.

Calculation:

- Express the proline content on fresh-weight-basis as follows:

$$\text{mmoles per g tissue} = \frac{\text{mg proline/mL} \times \text{mL toluene}}{115.5} \times \frac{5}{\text{gm sample}}$$

(*where 115.5 is the molecular weight of proline)

2.3. CHLOROPHYLL ESTIMATION

Chlorophylls and carotenoids were extracted by 80% acetone and assessed according to Lichtenthaler and Wellburn (1983). One gram of fresh leaf samples were collected from the control and the stress treated plants. The leaves were cut into small pieces and 20 ml of 80% acetone was added to it along with 500 mg MgCO₃. The extract was homogenized and maintained at 4 °C for about 4 hours. Later the samples were centrifuged at 1000 pm for 5 minutes and the resulting supernatant was transferred to a new tube and optical density was recorded at 645 nm (chlorophyll a) and 663 nm (chlorophyll b) and analysed for their respective concentration in control and stressed plant seedlings

Calculation

1. Chlorophyll A (mg lg):

$$12.7 (A_{663}) - 2.69 (A_{645}) \times V/1000 \times w$$

2. Chlorophyll B (mg lg):

$$22.9 (A_{645}) - 4.68 (A_{663}) \times V/1000 \times w$$

3. Total Chlorophyll (mg lg):

$$20.9 (A_{645}) - 8.02 (A_{663}) \times V/1000 \times w$$

A - Absorbance at specific wavelength (nm)

w - Fresh weight of the sample (g)

V - Volume of the sample (ml)

A - Length of the light path in the cell (1cm)

2.4. FOURIER TRANSFORM INFRARED (FTIR) ANALYSIS

The FTIR spectrum of solid sample was obtained from ethanolic extract of plant leaves powder. To run the solution, a drop of the extract was placed on the face of a highly polished salt plate (i.e. 100mg of potassium bromide, KBr). A second plate was placed on the top of the first plate so as to spread liquid in these layers between the dried plates and clamp the plates together in suitable fashion. Then the spectrum was obtained with the help of spectrophotometer and computer software attached with it⁵. The frequencies of different compounds present in the sample were analysed. The same procedure was followed for the suitable standard. The samples were run at infrared region between 400nm and 4000nm. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information for IR spectroscopic study.

3. RESULTS AND DISCUSSION

After 4 weeks of growth, the plant *Vigna radiata* exhibited some differences in terms of growth measurements. Especially the different concentrations such as (100ppm and 250ppm) of fertilizer (P) and salt (NaHCO₃) for various mung bean varieties CO6, CO(Gg) and traditional showed the greatest variations in number of leaf production, leaf surface area (width) and shoot height. The toxic effects observed on the leaves were mainly necrosis and losing chlorophyll mostly in leaves at high NaHCO₃ concentration. It was severe in

lower leaves than that of upper leaves. In this plant, the death of seedlings was observed to be preceded by reduction leaf area, necrosis and fall of leaves.

In the present study, the effect of NaHCO₃ stress on morphological characteristics such as percentage of germination, root length, shoot length of *Vigna radiata* were investigated. There was a large range of variation in germination percentage under NaHCO₃ salt conditions. It is inferred that as the NaHCO₃ concentration increased, the germination percentage declined (Table-1). The highest NaHCO₃ concentration used in this experiment was 250 ppm and 100 ppm. Similar results were reported in mung bean^{6,7}. The effect is more pronounced at higher salinity levels.

General symptoms of damage by salt stress can be attributed to the extent of growth inhibition, accelerated development, senescence and death during prolonged exposure. Growth inhibition is the primary injury that leads to other symptoms although programmed cell death may also occur under severe salinity shock. Salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells. As a result of stomatal closure, photosynthesis declines and photoinhibition and oxidative stress occur. An immediate effect of osmotic stress on plant growth is its inhibition of cell expansion either directly or indirectly through abscisic acid.

Moderate and high salinities negatively affected shoot length in all the varieties (Table 1). Leaf length significantly decreased at high salinity and a significant decrease in leaf number was observed in higher concentrations of NaHCO₃ treatments in the plant. In our study salinity had no significant effect on the leaf area, while treatment of plants with higher concentrations induced significant reduction of leaf area.

Table - 1 Seed germination of *Vigna radiata* against various treatments

No.	Treatment	Co6	Co (Gg)	Traditional	ppm	Total
I.	Organic fertilizer	-	-	50	-	45
II.	Inorganic fertilizer (P)	-	-	50	-	40
III.	Salt (Sodium bicarbonate)	50	50	50	100	35
IV.	Salt (Sodium bicarbonate)	50	50	50	250	30

While the chlorophyll content was considered i.e., total chlorophyll contents were significantly affected by variety type, salinity and their interactions. As shown in Table 2, the contents of total chlorophyll negatively affected in plant by moderate and high salinity treatments. Moderate and high salinities induced a significant increase in proline contents in the leaves and roots of *Vigna radiata*⁸.

In plants, salt stress is a critical factor that severely affects entire plant growth and metabolism. Salinity stress involves complex and variable mechanisms that related to different metabolic pathways of various organs. Growth has been considered as the result of different physiological mechanisms and its reduction after salt treatment has been widely described in different literature⁹. In both cultivars at low and moderate salinities showed no plant death after 15 days of culture, the plant death was observed for BS cultivar at salt 250 ppm CO6, salt 250 ppm CO(Gg) , salt 250 ppm traditional seed, salt 100 ppm CO6 , salt 100 ppm CO(Gg) , salt 100 ppm traditional seed NaHCO₃ in contrast to BZ. Such response could be attributed to higher degree of salinity tolerance in B2 cultivar. Sign of leaf necrosis has often been correlated with high sodium (or) chloride accumulation in leaves¹⁰.

The results showed a negative relationship between salinity stress and vegetative growth parameters such as leaf number, leaf area, and shoot length. However, the responses of cultivars were different. In a previous study, growth inhibition under severe salinity in pistachio has been attributed to a decrease in carbon assimilation due to stomatal limitation and/or metabolic impairment¹¹. Moreover, the reduction in plant growth under saline conditions has also been attributed to a result of direct inhibition of cell division and expansion¹².

The leaf length to shoot length ratio in BZ cultivar showed a significant increase in moderate and high salt concentrations but they showed no variations in the study plant. These findings suggest a higher water uptake capacity. The negative effect on plant water relations was induced by an increase in soluble salts which decelerate the uptake of water and nutrients causing osmotic effects and toxicity¹³.

Under salinity stress plants accumulate compatible solutes such as proline and soluble sugars which are known for their osmoprotection activity¹⁴. The accumulation of metabolites that act as compatible solutes is one of the common responses of plants to changes in the external osmotic potential¹⁵. Proline can be considered as a biochemical marker of salt stress level¹⁶.

Table 2. Effect of Organic fertilizer, Inorganic fertilizer (Potassium) and Salt (Sodium bicarbonate) on shoot and leaf length of *Vigna radiata*

No	Varieties of Mung bean	Concentrations (ppm)	Shoot length (cm)	Leaf length (cm)
1	CO6	-	17.7	2.3
2	CO(Gg)	-	20.5	2
3	traditional seed	-	22	3.5
4	Organic fertilizer	-	20	2.8
5	Inorganic fertilizer (P)	-	23	3.3
6	Salt (NaHCO ₃) CO6	100	15.8	2
7	Salt (NaHCO ₃) CO(Gg)	100	19	2.5
8	Salt (NaHCO ₃) (traditional seed)	100	25	2.8
9	Salt (NaHCO ₃) CO6	250	16.5	3.2
10	Salt (NaHCO ₃) CO(Gg)	250	19.5	1.9
11	Salt (NaHCO ₃) (traditional seed)	250	21	2

3.1. PROLINE TEST

These tests are showed that the various treatment of three varieties of *Vigna radiata* with measurements. Every pot contained 10 numbers of seeds. Average growth of the seed was taken as single value. After 10 days interval the measurement was taken and recorded for compare with other treatments. Fertilizers and salt concentration (100ppm and 250 ppm) in all verities of *Vigna radiata* are showing minimum growth while compare to the normal growth. Similarly salinity stress affects the development and underlying mechanisms such as seed germination, seedling growth and vigor, vegetative growth, flowering, and fruit set. A general decline in physical parameters in Lablab during salt stress in time- and concentration-dependent manner indicated that the salt stress above 100 mM significantly affects the growth potential. The decrease in growth can be attributed to the reduced cell elongation resulting from decreased turgor, cell volume, and cell growth, as has been observed by Boyer (1988). Contrary to salinity-stressed soybean¹⁷ and chickpea¹⁸, Lablab seedlings showed a moderate reduction in fresh and dry weights up to 300 mM. Observed changes in shoot and root lengths of Lablab were similar to those of salt-tolerant Lucerne¹⁹, and salt- and temperature stressed French bean²⁰. Nevertheless, salinity influenced Lablab shoot growth more negatively than root, similar to Medicago

citrina²¹, indicating the moderate tolerance of the plant. Reduced effect on the root growth could be due to expenditure of more photosynthetic energy on root growth in search of water and/or reducing water loss, thus maintaining higher water relations.

Proline has been implicated as antistress organic molecule, in some higher plants and it is known to accumulate in response to environmental stress²². In our study, proline accumulated in fresh material and that was estimated. It is quite interesting to note that the all varieties of *Vigna radiata* are grown in salt concentration (100ppm 250ppm). That showed some amount of free proline accumulation. It has also been reported that the plants grown under stress condition exhibit a remarkable increase in proline content in some legumes²³. Proline accumulation was normally observed during stress condition.

Increased accumulation of proline is to maintain intercellular osmoticum during stress condition. The higher magnitude of proline accumulation may help plants to tolerate the degradation by maintaining cell turgidity as recorded and may protect plants against induced damage. The accumulation of proline content under stress may be due to increased synthesis of protein bound proline²⁴.

3.2. CHLOROPHYLL ESTIMATION

The detailed chlorophyll estimation studies are shown in Fig -1 to Fig - 8. In control series, chlorophyll content is in the range cogg, co-6, control (traditional seed). Control traditional seed has more chlorophyll than the treated seedlings. Among organic and inorganic fertilizer, inorganic shows more chlorophyll content. The effect of salt concentration at 250ppm, the results more chlorophyll content in Co6 variety, in the range of CO-6 > CONTROL > TRADITIONAL SEED CoGG

Among salt concentration at 100ppm, Control traditional seed shows the higher content of chlorophyll than the varieties of Cogg and Co-6. Range is CONTROL TRADITIONAL SEED > CoGG > CO-6 . The CO-6 variety at 250ppm salt, it has more total CHL than salt 100ppm and control series, whereas inn CoGG and CONTROL at salt 250ppm has more Total chlorophyll than salt 100ppm and control series

Lower chlorophyll content recorded was control cogg variety. Highest chlorophyll content is present in inorganic fertilizer which is 0.0702 mg/g in total chlorophyll. Finally our result shows that the organic and inorganic shows more chlorophyll content than the others

Sequential order of our result is Organic & Inorganic > Salt 100ppm > CONTROL > Salt 250ppm

Fig –1. Chlorophyll test in control (traditional) for *Vignaradiata*

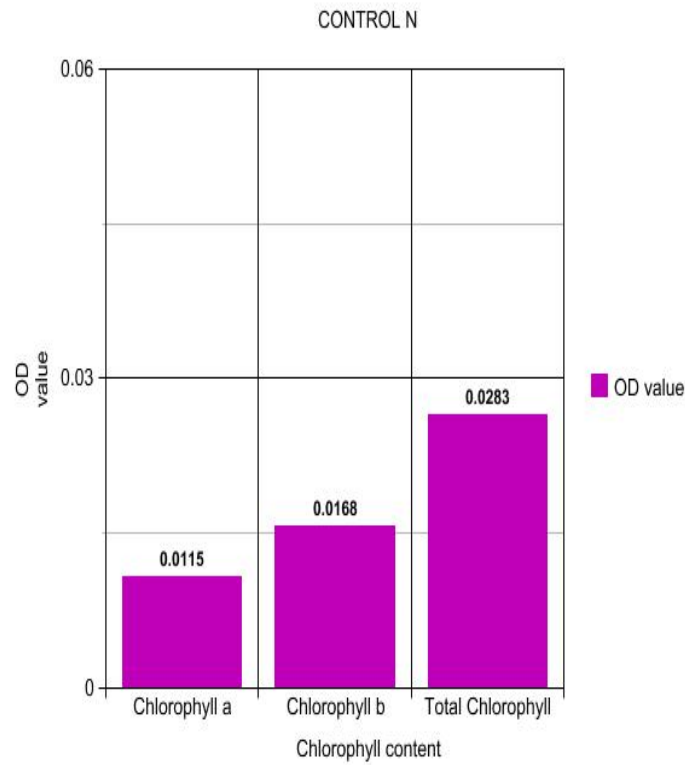


Fig –2. Chlorophyll test in control CO(GG) variety

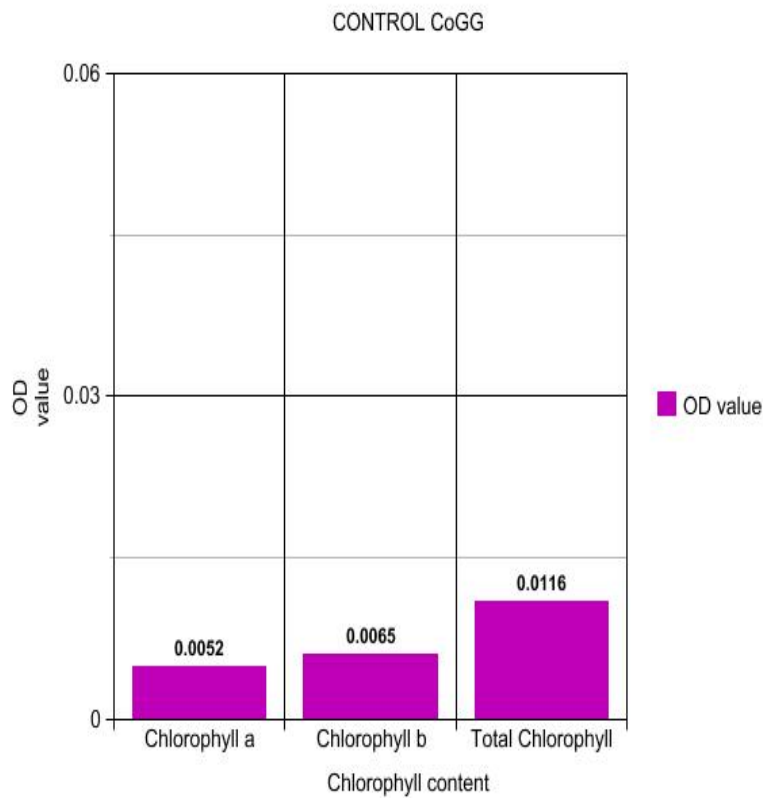


Fig –3. Chlorophyll test in *Vigna radiata* (Organic)

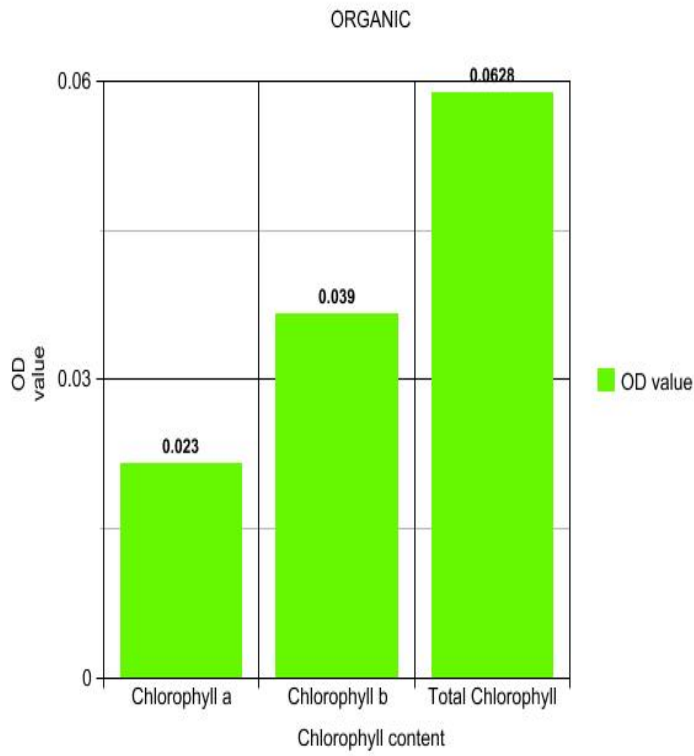
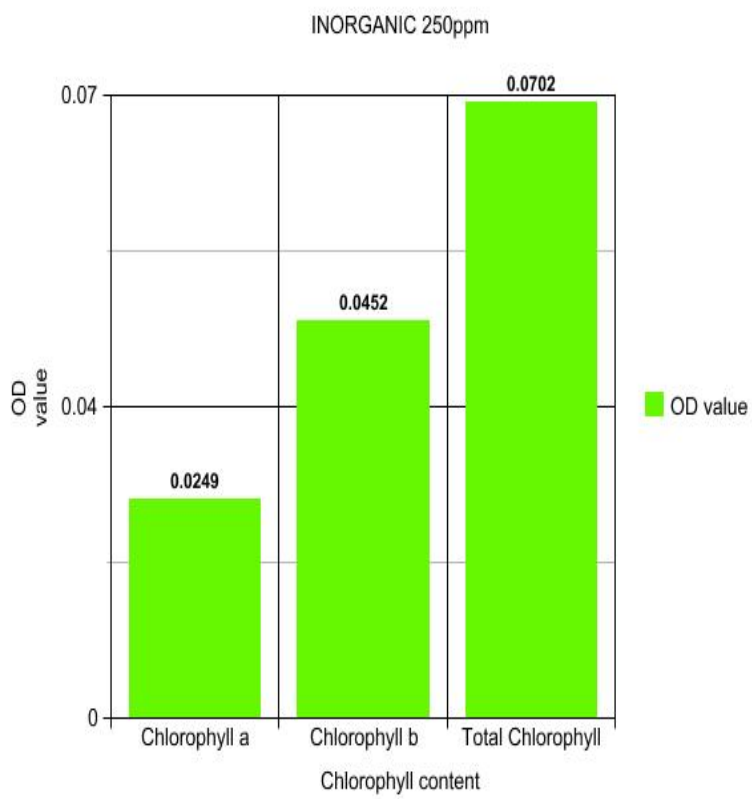


Fig –4. Chlorophyll test in *Vigna radiata* (Inorganic)



S

Fig – 5. Chlorophyll test analysis in NaHCO₃ salt treated variety CO6 (*Vigna radiata*)

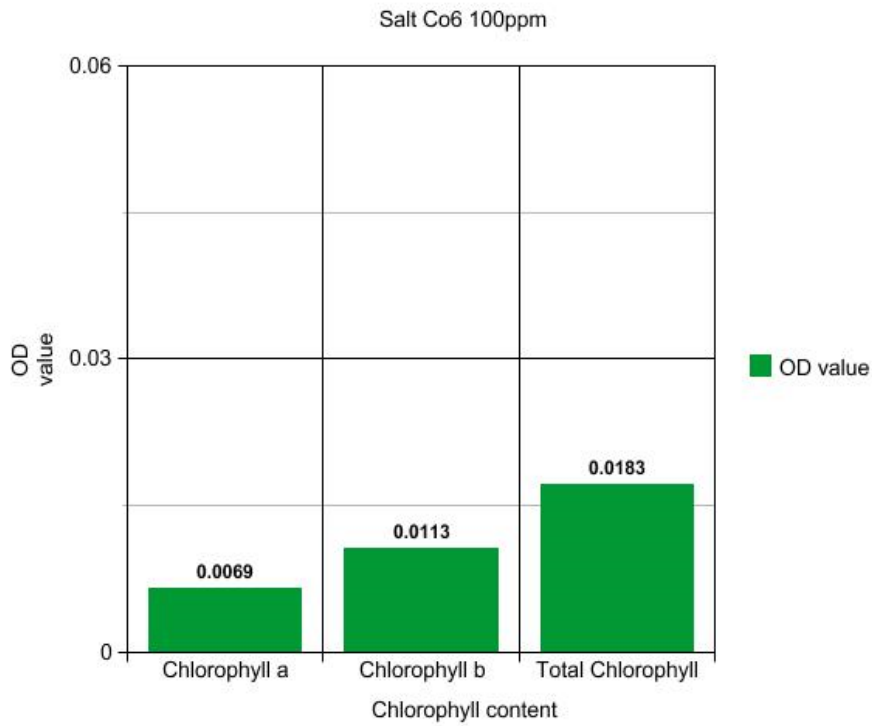


Fig-6. Chlorophyll test analysis (NaHCO₃ salt treated in traditional seed) (*Vigna radiata*)

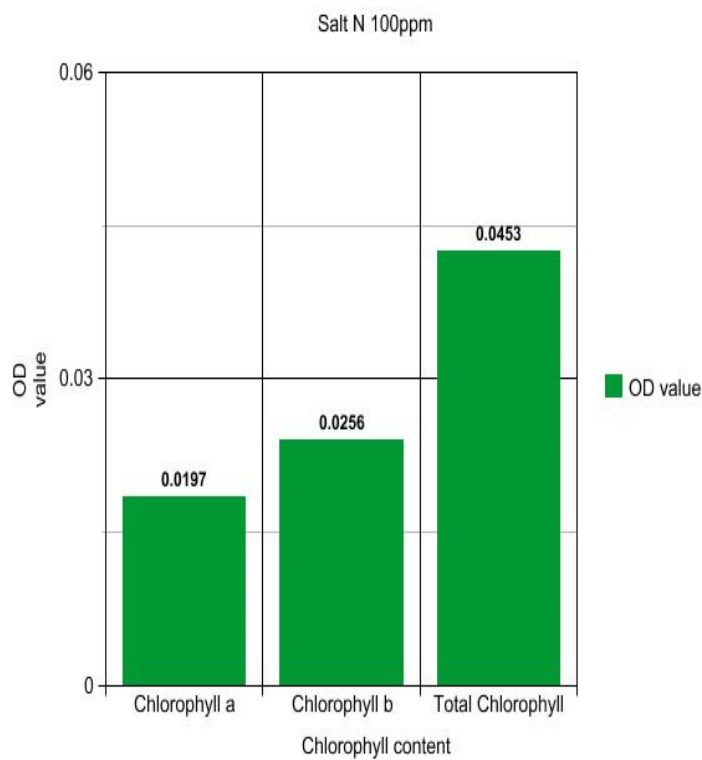
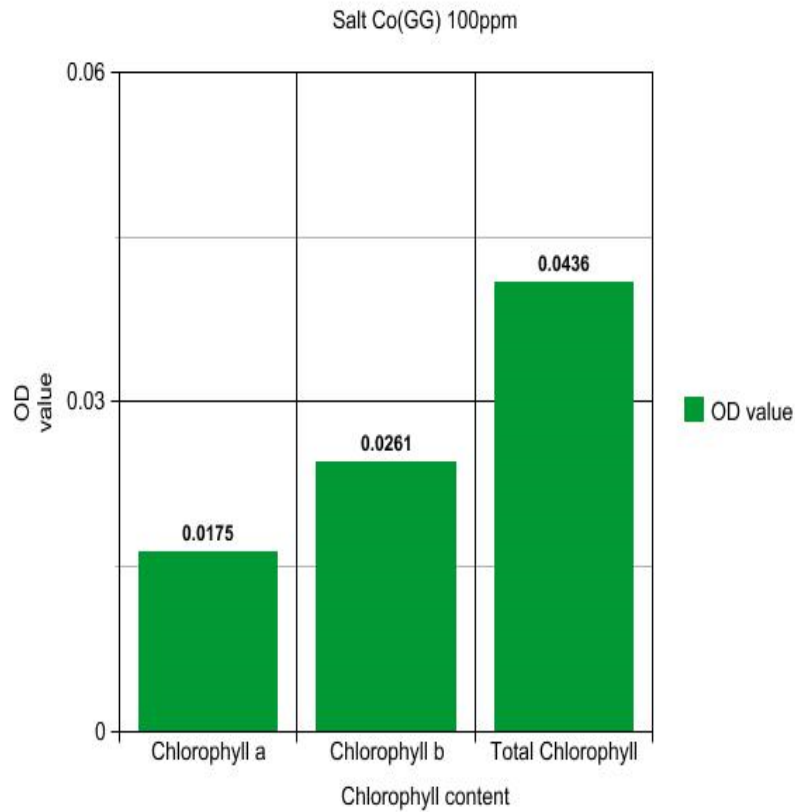
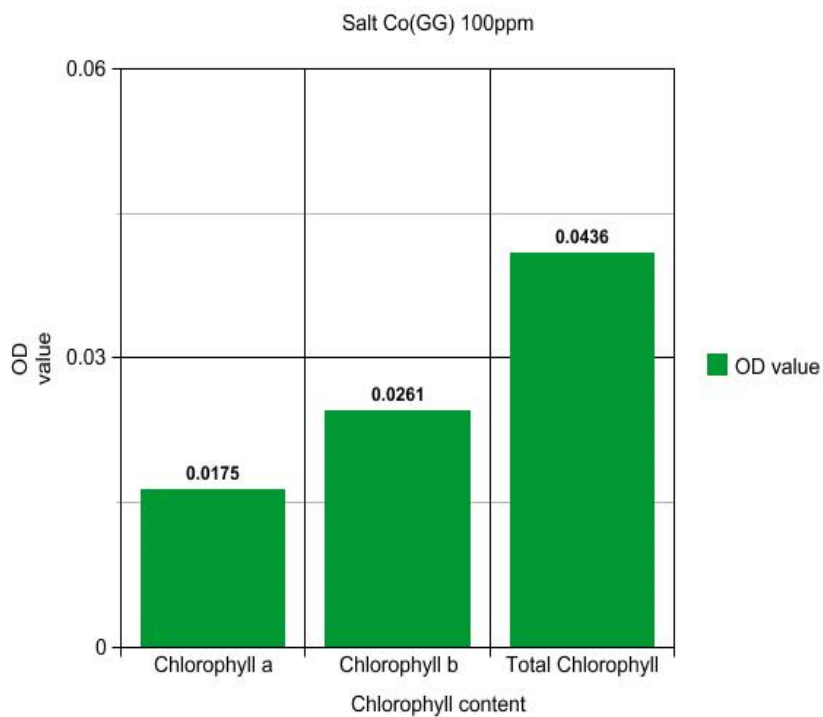


Fig –7. Chlorophyll test analysis (NaHCO₃ salt treated in CO(Gg) (*Vigna radiata*))Fig –8. Chlorophyll test analysis (NaHCO₃ salt treated in CO (Gg)) (*Vigna radiata*)

3.4. FTIR ANALYSIS

FTIR spectroscopy was useful for identification of functional groups, when run under IR region from 400-4000cm⁻¹. There was a variation in the peaks of the sample which helps to determine the functional

groups. Each peak represents single compound based on their density. The typical IR spectra for *Vigna raddiata* extract are obtained and shown (Table 3, Figure -9 to Fig - 12).

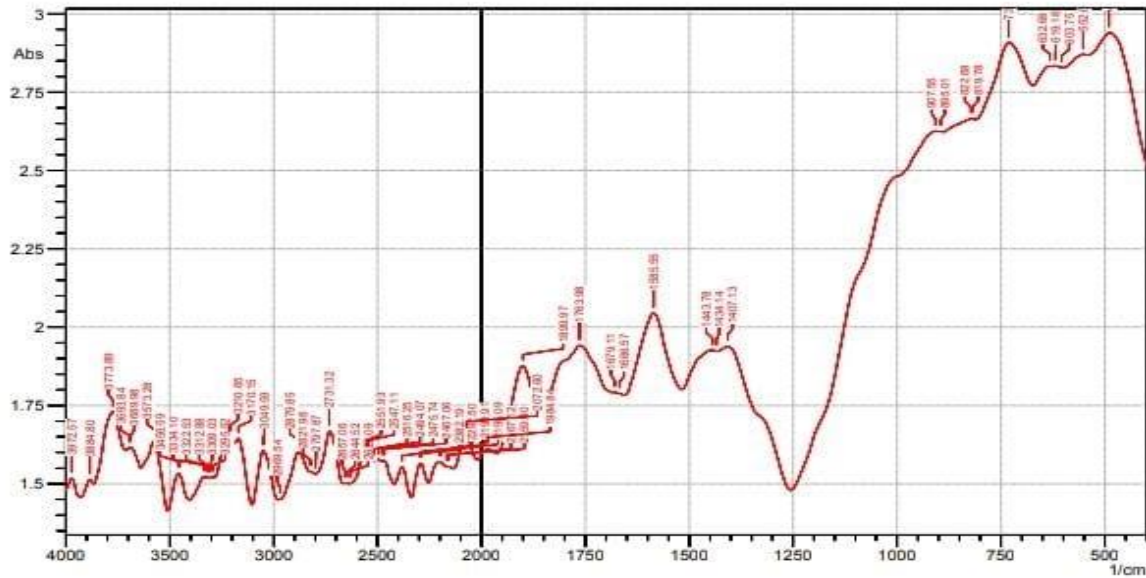
Figure 9 represents the IR spectrum of *Vigna radiata* leaf (CONTROL). In this spectrum 9 peaks are identified that are 731.05, 907.55, 1443.78, 1679.11, 1984.84, 2494.07, 2969.54, 3456.59, 3972.57. Figure 10 represents the IR spectrum of *Vigna radiata* (Salt 100 ppm). In this spectrum 10 peaks are identified that are 712.73, 994-35, 1009.78, 1421-6, 1591.34, 1906.72, 2457.42, 2933-85, 3444-05, 3973-53.

Figure 11 shows the IR spectrum of *Vigna radiata* (Salt 250 ppm). In this spectrum 9 peaks are identified that 594-1, 1007.85, 1415.81, 1719-61, 1995.45, 2462-24, 2952.18, 3453-69, 3737.24. Figure 12 shows the IR spectrum of *Vigna radiata* (Inorganic fertilizer). In this spectrum 9 peaks are observed that 701.015, 976.99, 1.96, 1410.02, 1729, 26, 2355.19, 2930.96, 3449.84, 3938.81.

In all varieties, IR spectrum showed the broad absorption around 3797.84 cm^{-1} and 2353.16 cm^{-1} the O-H stretching frequency is due to the presence of alcohols or phenols. Another prominent absorption around 2918.30 cm^{-1} and the weak absorption such as 1371.39 cm^{-1} , 1325.10 cm^{-1} , is mainly due to alkanes (C-CH₃ stretching vibrations). In the spectrum, the medium range such as 1618.28 cm^{-1} , 1631.78 cm^{-1} , is mainly due to amines (C=C stretching vibration). The C=O stretching vibrations at 1728.22 cm^{-1} , due to the presence of esters or carboxylic acid or lactones. At 1442.75 cm^{-1} , CH bending frequency is due to the aldehyde or ketones. Particularly below 1200 cm^{-1} , C-O-C vibrations in ester at the range between 1100 cm^{-1} to 1200 cm^{-1} due to the presence of acetates or formates. At the range between 600 cm^{-1} to 700 cm^{-1} , -CH-CH- bond frequency, is mainly due to the ethylene groups.

Similarly FTIR spectrum analysis of *Caralluma fimbriata* revealed the peak value at 2970.38 and 2885.51 cm^{-1} refers to the presence of alkanes (C-H stretch). The peak at 1759.08 and 1666.50 cm^{-1} corresponds the carboxylic acid group (C=O stretch). A peak of 1327.03 cm^{-1} showed the presence of aromatic amines (C-N stretch). The peaks of 1273.02, 1087.85 and 1049.28 cm^{-1} indicate the alcohols, carboxylic acids, esters, ethers (C-O stretch). A peak of 879.54 cm^{-1} revealed the alkenes (=C-H bend). This basic reports are used to find out the presence of phenols, alkanes and aromatic amines^{25,26}. Same absorption spectrums are obtained in extract of *Aerva lanata*²⁷ and ethanolic extracts of *Albizia lebbek*²⁸.

Fig –9. .FTIR analysis for control



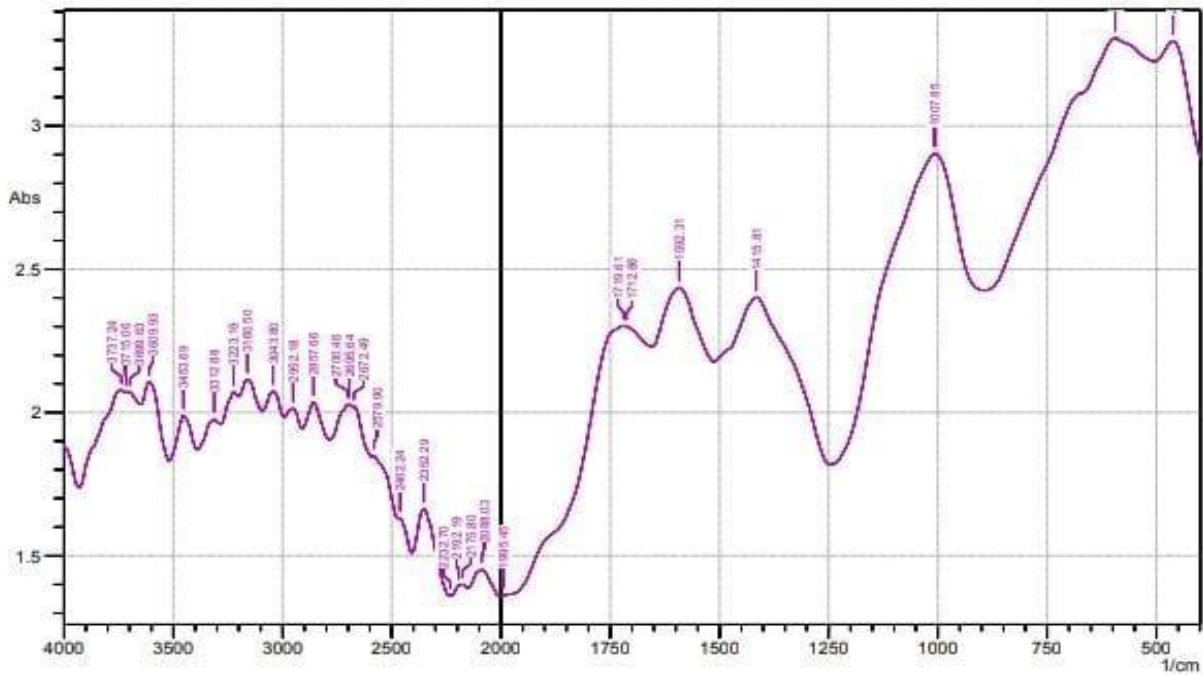
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	489.94	2.94	0.1997	542.02	399.28	402.6077	18.383
2	552.63	2.8713	0.0102	597.96	542.98	156.9269	0.3447
3	603.75	2.8291	0.0003	605.67	598.92	19.0975	0.001
4	619.18	2.8339	0.0027	625.93	605.67	57.3607	0.0308
5	632.68	2.8337	0.0091	671.26	626.89	124.7266	0.3808
6	731.05	2.908	0.182	811.1	672.22	389.6263	12.15
7	819.78	2.6655	0.0003	820.75	812.07	23.1255	0.0018
8	822.68	2.6656	0.0014	887.29	820.75	176.1367	0.1485
9	895.01	2.6242	0.0002	895.97	888.26	20.2433	0.001
10	907.55	2.6258	0.0386	1254.75	895.97	772.2802	36.6476
11	1407.13	1.9389	0.0728	1429.31	1255.71	302.6003	7.6612
12	1434.14	1.924	0.0005	1436.07	1430.28	11.1318	0.0022
13	1443.78	1.9263	0.0127	1517.08	1437.03	150.9749	1.8084
14	1585.55	2.0452	0.2529	1654.03	1518.04	260.4267	16.6999
15	1666.57	1.7878	0.001	1668.5	1655	24.1053	0.0055
16	1679.11	1.7922	0.0023	1685.86	1669.46	29.3626	0.0234
17	1763.98	1.9412	0.1737	1855.6	1686.82	314.1658	16.2114
18	1899.97	1.8759	0.1928	1961.69	1859.46	180.1916	9.558
19	1984.84	1.6111	0.0218	2016.66	1962.65	86.334	0.5945
20	2072.6	1.6612	0.097	2135.29	2021.49	182.9201	5.0703
21	2159.4	1.5556	0.0017	2162.3	2137.22	38.9213	0.0255
22	2167.12	1.5555	0.0002	2168.08	2163.26	7.4983	0
23	2195.09	1.5699	0.0005	2196.05	2170.01	40.6502	0.0016
24	2199.91	1.5708	0.0043	2253.92	2197.02	88.0185	0.5721
25	2292.5	1.5649	0.0836	2335.9	2254.88	123.6	3.7129
26	2382.19	1.5535	0.0746	2419.8	2336.86	125.5847	3.0991
27	2467.06	1.5733	0.0047	2469.95	2420.77	75.5647	0.1586
28	2475.74	1.5739	0.0011	2479.6	2470.92	13.6583	0.0061
29	2494.07	1.5777	0.0009	2495.99	2484.42	18.2338	0.007
30	2516.25	1.5743	0.0004	2518.18	2513.36	7.5904	0.0011
31	2547.11	1.601	0.0011	2548.08	2519.14	45.8777	0.0182
32	2551.93	1.6015	0.0017	2562.54	2549.04	21.6007	0.0156
33	2629.09	1.5023	0.001	2635.84	2626.2	14.4841	0.0078



34	2644.52	1.5025	0.0018	2650.31	2638.73	17.3793	0.0095
35	2657.06	1.5034	0.0018	2661.88	2651.27	15.9393	0.0086
36	2731.32	1.6681	0.1515	2794.01	2667.67	200.7915	9.2117
37	2797.87	1.5321	0.0005	2800.76	2794.97	8.864	0.0015
38	2821.98	1.5396	0.0026	2826.8	2801.73	38.523	0.0359
39	2879.85	1.6019	0.0963	2965.68	2827.77	212.45	6.2459
40	2969.54	1.4519	0.0012	2975.33	2966.65	12.5968	0.0075
41	3049.59	1.605	0.1653	3103.6	2978.22	191.2636	10.6139
42	3170.15	1.6455	0.0563	3187.51	3104.56	129.439	2.5965
43	3210.65	1.6495	0.0449	3282.02	3188.47	150.3214	2.8517
44	3295.52	1.5204	0.0007	3297.45	3291.67	8.7952	0.0021
45	3309.03	1.5198	0.0001	3309.99	3303.24	10.258	0.0004
46	3312.88	1.5201	0.0003	3318.67	3311.92	10.2615	0.0019
47	3322.53	1.5201	0.0006	3326.39	3319.63	10.2607	0.0021
48	3334.1	1.5222	0.0088	3402.58	3327.35	112.0241	0.3768
49	3456.59	1.5332	0.1024	3509.63	3403.54	157.3156	5.5106
50	3573.28	1.636	0.1552	3637.9	3510.6	198.509	9.9372
51	3689.98	1.6156	0.0013	3690.95	3638.87	82.695	0.3075
52	3693.84	1.6161	0.0019	3708.31	3690.95	27.9982	0.0202
53	3773.89	1.7297	0.0018	3774.85	3709.27	108.9625	0.1599
54	3884.8	1.514	0.0267	3933.02	3864.55	101.9715	0.7545
55	3972.57	1.5179	0.0484	4000.54	3933.99	99.2543	1.5877

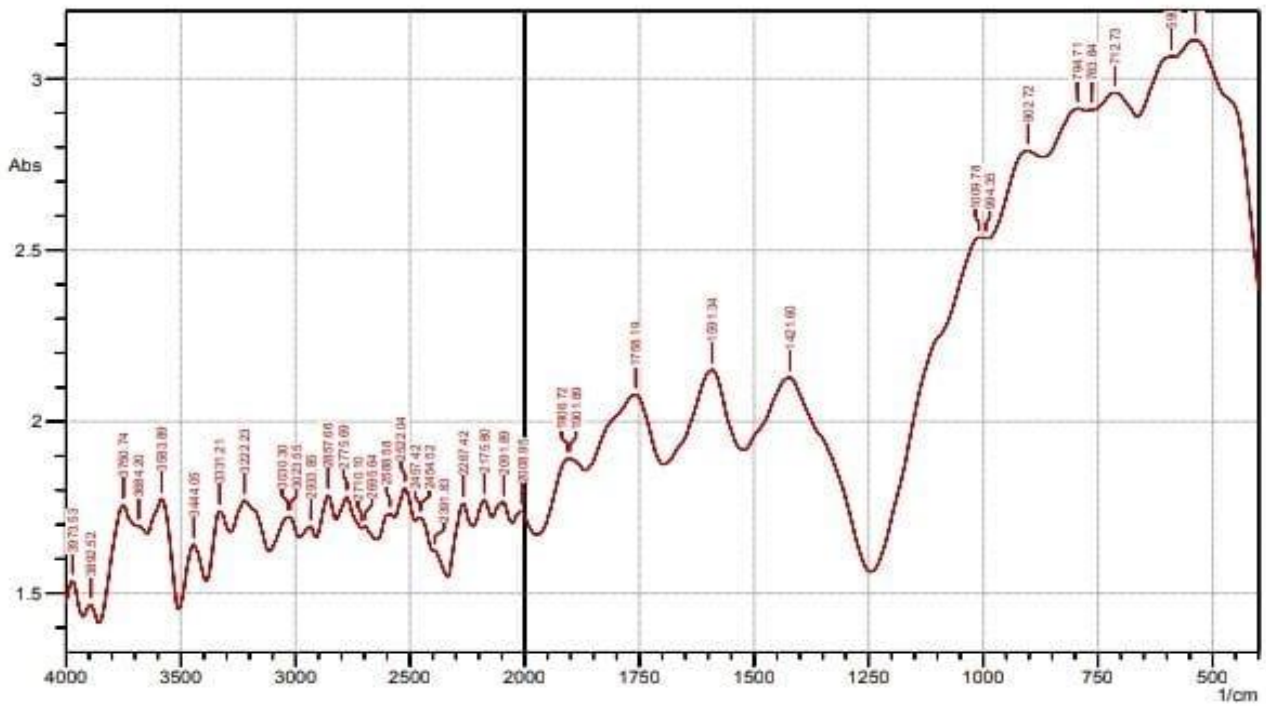


Fig –11. FTIR analysis in sodium bicarbonate salt 250 ppm (*Vigna radiata*)



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	461.97	3.2954	0.2047	503.44	399.28	330.6323	12.4651
2	594.1	3.3074	0.1419	668.36	506.34	525.6219	11.925
3	1007.85	2.9033	0.6734	1247.03	894.04	868.5435	119.6771
4	1415.81	2.4028	0.3572	1512.26	1248	578.6238	51.0322
5	1592.31	2.4355	0.2297	1653.07	1513.22	324.8461	16.8793
6	1712.86	2.2999	0.0013	1713.83	1654.03	135.3886	0.1311
7	1719.61	2.3022	0.0186	1992.55	1714.79	487.1922	4.1983
8	1995.45	1.3618	0.001	2006.05	1993.52	17.057	0.0091
9	2088.03	1.453	0.0779	2147.83	2010.88	193.9707	5.9315
10	2175.8	1.3997	0.0059	2187.37	2148.79	53.8131	0.1084
11	2192.19	1.3964	0.0029	2225.95	2189.3	50.5891	0.069
12	2232.7	1.3622	0.0009	2237.52	2226.91	14.4468	0.0053
13	2352.29	1.6628	0.2026	2408.23	2238.49	260.4671	16.9722
14	2462.24	1.63	0.0043	2464.17	2409.19	86.684	0.5314
15	2579.9	1.8487	0.0207	2589.55	2470.92	209.393	3.5074
16	2672.49	2.0206	0.0048	2674.42	2593.4	156.1826	0.4062
17	2695.64	2.0284	0.0007	2696.6	2678.28	37.0946	0.0102
18	2700.46	2.0284	0.0045	2780.51	2697.56	163.772	0.7246
19	2857.66	2.0358	0.1076	2907.81	2781.47	249.3253	6.237
20	2952.18	2.0134	0.0133	2959.9	2908.78	101.4036	0.4486
21	3043.8	2.0737	0.0792	3093.95	2994.62	202.3589	4.2358
22	3160.5	2.1144	0.0034	3162.43	3094.92	139.3506	0.4727
23	3223.19	2.072	0.0392	3279.13	3203.9	152.9712	1.7996
24	3312.88	1.9774	0.0437	3384.25	3282.99	196.5812	2.5661
25	3453.69	1.9887	0.1382	3519.28	3390.04	247.979	8.8402
26	3609.93	2.1068	0.0031	3610.9	3520.24	179.0376	1.2881
27	3699.63	2.0716	0.0018	3701.56	3651.41	102.8083	0.0617
28	3715.06	2.0707	0.0007	3722.77	3713.13	19.9677	0.005
29	3737.24	2.0785	0.0306	3930.13	3723.74	400.7236	7.6732

Fig –12. FTIR analysis in sodium bicarbonate salt 100 ppm (*Vigna radiata*)



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	537.2	3.1123	0.0205	541.06	399.28	410.3456	21.3771
2	590.24	3.0658	0.0189	665.47	582.53	248.5332	1.7006
3	712.73	2.9589	0.0595	759.99	666.43	274.3169	3.0761
4	763.84	2.9101	0.0014	776.38	760.95	44.8818	0.0098
5	794.71	2.9131	0.0318	869.93	777.35	264.3764	1.6322
6	902.72	2.79	0.0816	989.53	870.9	320.2686	5.5504
7	994.35	2.5354	0.0001	995.31	990.49	12.2254	0.0003
8	1009.78	2.5372	0.0437	1246.07	999.17	517.9945	13.4961
9	1421.6	2.129	0.3408	1521.9	1247.03	529.6974	51.5881
10	1591.34	2.1515	0.2503	1696.47	1522.87	348.2921	18.9836
11	1758.19	2.0786	0.2091	1868.14	1697.43	337.9495	19.2165
12	1901.89	1.8923	0.001	1902.86	1869.1	63.3903	0.1104
13	1906.72	1.8927	0.0095	1974.23	1903.82	125.1202	0.4606
14	2008.95	1.7387	0.0061	2011.84	1975.19	62.4311	0.0947
15	2091.89	1.7644	0.0017	2092.86	2054.28	66.8169	0.0538
16	2175.8	1.7706	0.0584	2224.98	2143.97	140.9134	2.4327
17	2267.42	1.7614	0.123	2333.97	2225.95	182.4323	7.2395
18	2391.83	1.624	0.0063	2396.66	2334.93	97.9655	0.2204
19	2454.52	1.7199	0.0034	2456.45	2397.62	98.4766	0.3481
20	2457.42	1.7197	0.0004	2477.67	2456.45	36.4199	0.0208
21	2522.04	1.8052	0.0896	2568.33	2478.63	157.9809	4.0915
22	2588.58	1.7317	0.0061	2597.26	2569.29	48.328	0.1064
23	2695.64	1.6947	0.0117	2707.21	2649.34	97.0538	0.2616
24	2710.1	1.69	0.0003	2713	2708.17	8.1492	0.0009
25	2775.69	1.7806	0.0758	2821.98	2713.96	187.59	3.6515
26	2857.66	1.7853	0.0906	2909.74	2822.94	150.6101	3.9472
27	2933.85	1.6937	0.0014	2934.82	2910.71	40.4789	0.0463
28	3023.55	1.7215	0.0015	3024.51	2980.15	74.9742	0.0863
29	3030.3	1.7227	0.002	3036.09	3026.44	16.6037	0.0087
30	3222.23	1.7703	0.112	3282.02	3116.14	284.9856	11.1845
31	3331.21	1.7389	0.1215	3390.04	3286.84	172.153	6.2472
32	3444.05	1.6422	0.1432	3508.67	3391	184.9507	9.0159
33	3583.89	1.7746	0.2019	3645.62	3509.63	227.4352	15.0595

34	3684.2	1.6957	0.0023	3688.05	3646.58	69.9187	0.0958
35	3750.74	1.7569	0.1648	3857.8	3689.02	276.4833	14.1327
36	3892.52	1.466	0.0437	3925.31	3858.76	96.2111	1.5755
37	3973.53	1.5348	0.0783	4000.54	3926.27	110.8059	3.0506

Table 3. Identification of Functional groups through FTIR analysis

PEAKS	CONTROL (N)	SALT 100 PPM	SALT 250 PPM	INORGANIC 250 PPM	STRETCHING (OR) BOND	FUNCTIONAL GROUP
500-750	731.05	712.73	594-1	701.015	C-Br stretching vibration (OR) C-H bend stretching vibration (OR) N-H wag stretching vibration	presence of alkyl halides (OR) presence of alkynes (OR) presence of primary, secondary amines
750-1000	907.550	994-35	0	976.99	N-H wag stretching vibration	presence of primary, secondary amines
1000-1250	0	1009.78	1007.85	1031.96	C-N stretch stretching (OR) C-H wag (-CH ₂ X) stretching (OR) C-O stretching vibration	presence of aliphatic amines (OR) presence of alkyl halides (OR) presence of alcohols, carboxylic acids, esters, ethers
1250-1500	1443.78	1421-6	1415.81	1410.02	N-O stretching vibration (OR) C-C stretching vibration (OR) C-H bend stretching vibration (OR) N-O asymmetric stretching vibration	presence of nitro compounds (OR) presence of aromatics (OR) presence of alkenes (OR) presence of nitro compounds
1500-1750	1679—11	1591.3	1719-61	1729.26	N-O asymmetric stretching (OR) -C=C- stretching vibration (OR) C=O stretch	presence of nitro compounds (OR) presence of alkenes (OR) presence of aldehydes
1750-2000	1984-84	1906.72	1995.45	0	C=O stretch	presence of ketones

						(OR) Esters (OR) Anhydrides
2000-2500	2494.07	2457.4	2462-24	2355.19	C≡C stretch (OR) C≡N stretch	Alkynes (OR) Nitriles
2500-3000	2969.54	2933-85	2952.18	2930.96	O-H stretching (OR) C-H stretching vibration	presence of carboxylic acids (OR) presence of alkenes
3000-3500	3456-59	3444-05	3449.84		O-H stretching vibration	presence of alcohols, phenols
3500-4000	3972.57	3973-53	3737.2	3938.81	O-H stretching	alcohol

4. CONCLUSION

The demand for water has increased due to stress related issues like pollution. There is a significant drought prone conversion of agricultural lands worldwide leading to food shortages. In regard salt stress is one such major reason for failure of agriculture. This study tried to understand the extent of fertilizers and salt stress on some of the parameters analysis like chlorophyll estimation, proline and FTIR analysis to identify the best variety in *Vigna radiate*.

The salt (NAHCO₃) was used and its effect at different concentrations were analysed and it was found that chlorophyll content decreased and on the other hand proline contents accumulation increased or pigment loss due to unstable Na, K ion potential in the leaf due to salt stress influenced photosynthesis production. The resulting necrosis of leaves is attributed to it. On the other hand proline content accumulation is due to stress induced physiological reaction of the metabolites in the plant. The study can be extended to detailed phytophysiological analysis and control mechanism in the future.

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