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EFFECT OF SODIUM NITRATE NANOPARTICLES ON GERMINATION OF FINGER MILLET (*ELEUSINE CORACANA* L.) AND STUDY OF BIOCHEMICAL PARAMETERS IN GERMINATED FINGER MILLET (*ELEUSINE CORACANA* L.) SEEDS TREATED WITH SODIUM NITRATE NANOPARTICLES

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Abstract:

Sodium Nitrate nanoparticles are used to study the biochemical parameters like carbohydrate concentration, protein concentration, antioxidant scavenging capacity, catalase activity and germination capacity in finger millet seeds after treatment with nitrate nanoparticles. Total chlorophyll content and chla, chlb is measured after testing the germination capacity in soil. Carbohydrate content, protein content is found to be highest in 2mMNP treated group of 0.24mg/0.5mg/ml and 95mg/ml. Lipid mobilisation is higher in 2mMNP treated group compared to1mM and control which may be due to metabolic stress and lower metabolic rate in that treated group.Total reducing sugar content is recorded highest in 1mMNP and control groups than the total carbohydrate content may be due to synthesis of glucogenic compounds by breakdown of protein. Antioxidant scavenging capacity was found to be highest in control group (100%) followed by 1mMNP (98.6%) with slighter lesser value in 2mM treated group (95.9%).Catalase activity is recorded as high with 2mM treated group of 21.4% compared to 1mM and control with reference to standard which indicates oxidative stress. Total chlorophyll content , chla and chlb values are recorded as highest in 1mMNP treated group group (Chla: 8.6353, Chl b: 6.7363, Total chl: 22.0666) compared to control(Chla: 0.8976, Chl b: 2.7750, Total chl: 5.2846) and 2mMNP treated groups (Chla: 3.7264, Chl b: 3.0321, Total chl: 9.3794).

Key words: Carbohydrate concentration, Protein concentration, Antioxidant scavenging capacity, Catalase activity and Germination capacity.

Introduction:

Finger Millet (*Eleusine coracana* L.) is a minor cereal well known for its health benefits majorly due to its dietary fibres and poly phenol content. This crop is recognised as one of the staple foods in India especially in low-income groups [(1)]. Finger millet seeds contain exceptionally high amounts of calcium (>300 mg/100 g) and minerals, vitamins, and phytochemicals that include phenolic compounds associated with several potential health benefits [(2)].

Finger millet helps in losing weight, bone strengthening, antidiabetic, possess antiaging properties, regulate Hypertension, improve haemoglobin content and promotes health in children. Seeds of finger millet is rich in dietary fibres (18%), phytates (0.48%), protein (6%–13%) minerals (2.5%–3.5%), and phenolics (0.3%–3%)[(3)]. However finger millet seeds are also richest source for vitamins like thiamine, riboflavin, minerals like iron, methionine, isoleucine, leucine, phenylalanine and other essential amino acids[(3)].

In this study we have formulated Sodium nitrate nanoparticles that can increase the bioavailability of nitrate source to the millet and the nanoparticle formulation can also support the sustained release of the nitrate and helps in preventing the dissolution of the nitrate in to the environment there by improving the crop yield.

Methodology:

1. **Synthesis of Nitrate Nanoparticles:**

1mM and 2mM concentrations of NaOH and NANO3 is used to synthesize Nitrate nanoparticles and 1% PEG is used as a stabilizer to prevent aggregation and size reduction in nanoparticles. The chemicals are dissolved by continuous stirring and allowed to stand at room temperature for 15 days and characterized using UV- Visible spectrophotometer on 5th day and 15th day.

2. Germination set up of Pearl millet seeds:

Petri plates and Whatman filter paper no:1 are sterilized using autoclave and finger millet seeds are surface sterilized with 1% Sodium hypochlorite solution after overnight soaking and air drying. Seeds are placed randomly on the Petri dish covered with Whatman filter paper no:1 and spargelled with 1mM and 2mM nitrate nanoparticles and kept in dark conditions for overnight.

3. Estimation of carbohydrate by Anthrone method:

Carbohydrate estimation was carried out using procedure from David T. Plummer (1990)

4. Estimation of Protein by Biuret method:

Protein content was estimated using biuret method from Laboratory Manual in Biochemistry by J. Jayaraman

5. Estimation of reducing sugar by DNS method:

Reduced sugar content was estimated using procedure from Laboratory Manual in Biochemistry by J. Jayaraman

6. Catalase assay:

Catalase assay was performed according to Mahmoud Hussein Hadwan (2018)

7. Antioxidant assay using H2O2 scavenging method:

Antioxidant activity is performed using protocol of Gupta et al., (2022)

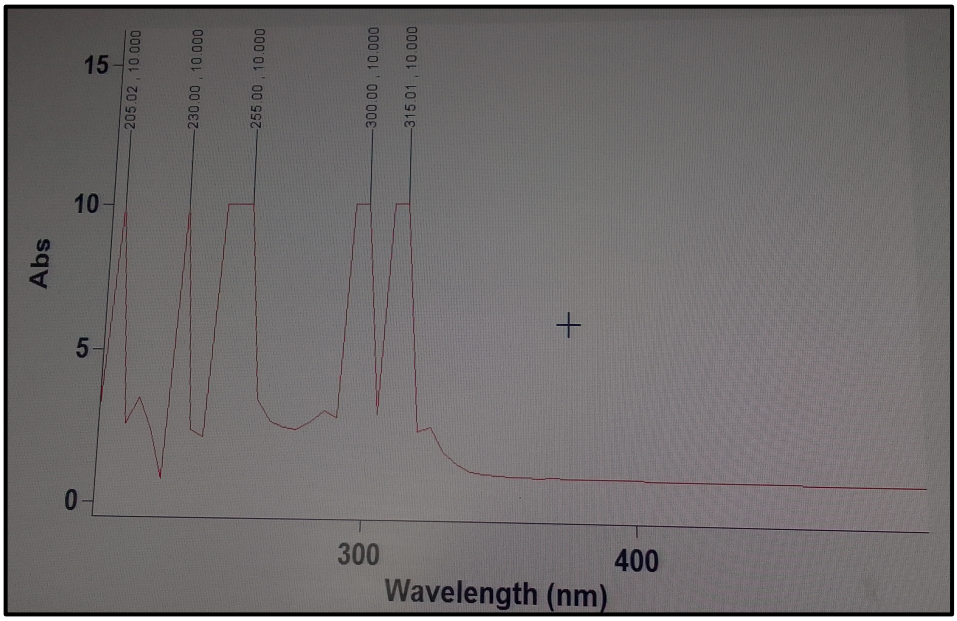
8. Germination setup using Pot technique:

Pots are bought from the market and covered with the soil and germination mixture in1:1 ratio and the seeds are transferred to pots. After seed transferring the pots are sprinkled with 1mM and 2mM nitrate nanoparticles and after definite growth of plant-lets the leaves are harvested and estimated for chlorophyll a, Chlorophyll b and total chlorophyll content.

9. Estimation of chlorophyll content:

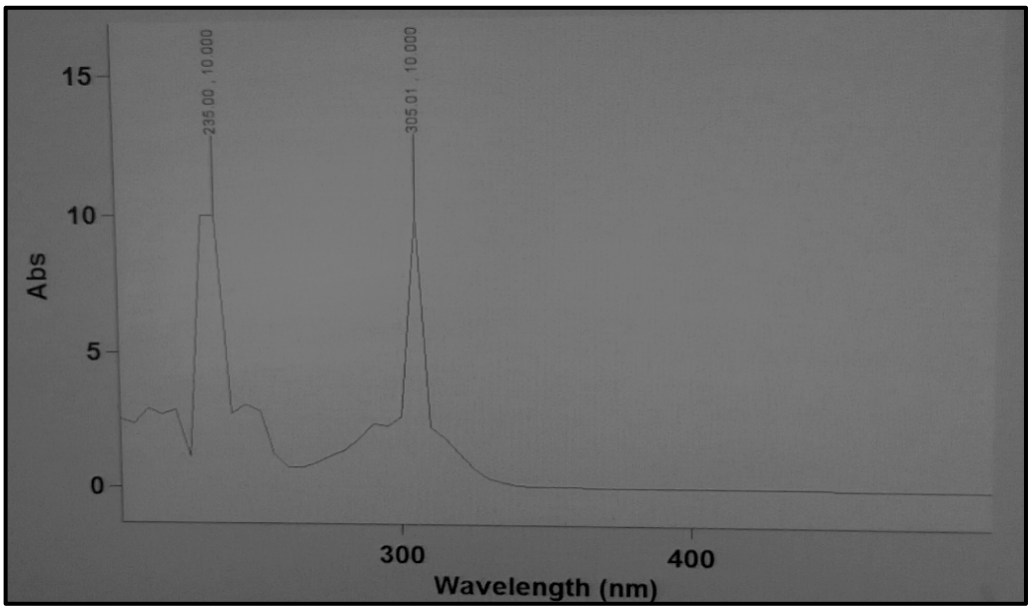
Chlorophyll content was estimated using procedure of Patricio MP et al., (2018)

Results:

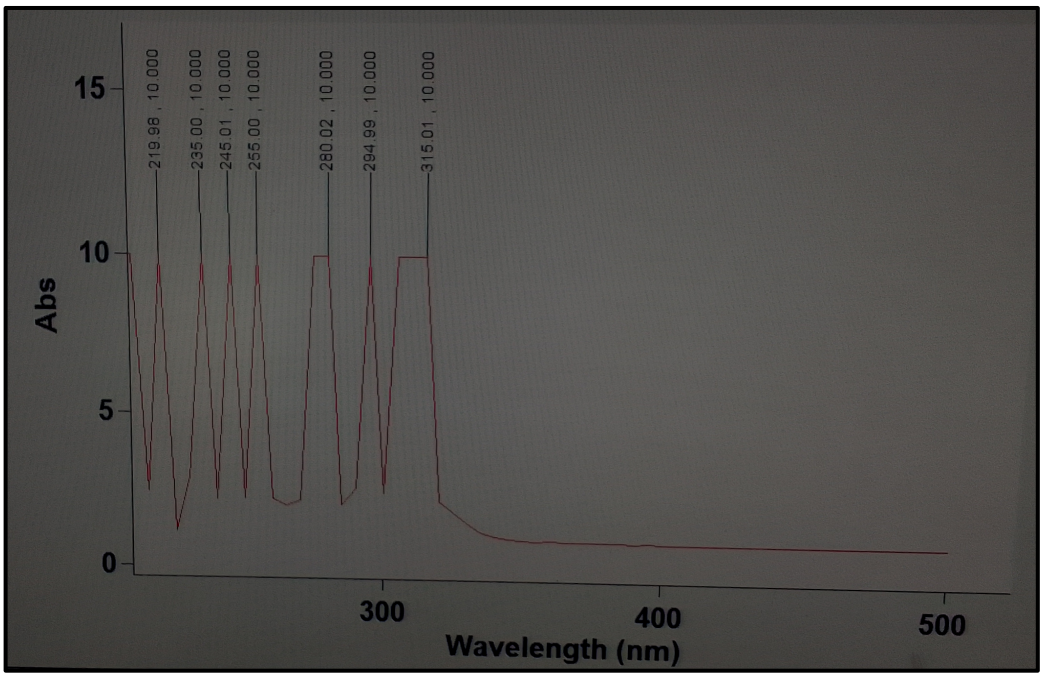


Scan:1: U.V Scan of Sodium Nitrate nanoparticles of 1mM in the wavelength range of 200-600nm. Nitrate ions absorb at wavelength 220nm resulted in shifting of wavelength to higher (Hyperchromic) or lower (Hypochromic) wavelengths.

Sodium nitrate nanoparticles are synthesised using 1mM and 2mM nanoparticles using NaOH and KNO_3 and tested for effect of nanoparticles on growth of finger millet plantlets. U.V visible scan is run after 5^{th} and 15^{th} day of preparation of nanoparticles with in wavelength range of 200- 600nm. With 1mMNPs characteristic peaks were observed at 235nm and 305nm wavelength range. Nitrate ions form characteristic peak at 220nm and formation of nanoparticles usually result in shifting of wavelength to either higher wavelengths (Hyperchromic shift) or to lower wavelengths (Hypochromic shift) and peak broadening effects observed with 1mMNPs and to some extent in 2mMNPs. 3mMNPs resulted in formation of a greater number of impure component peaks other than nitrate NPs and hence`` discarded from the study.



Scan:2 : U.V Scan of Sodium nitrate nanoparticles of 2mM in the wavelength range of 200-600nm. Characteristic peaks recorded at 235nm and 305nm



Scan: 3: U.V Scan of Sodium nitrate nanoparticles of 3mM in the wavelength range of 200-600nm. Characteristic peaks recorded at 219nm, 235nm,245nm,255nm,280nm,294nm and 315nm.

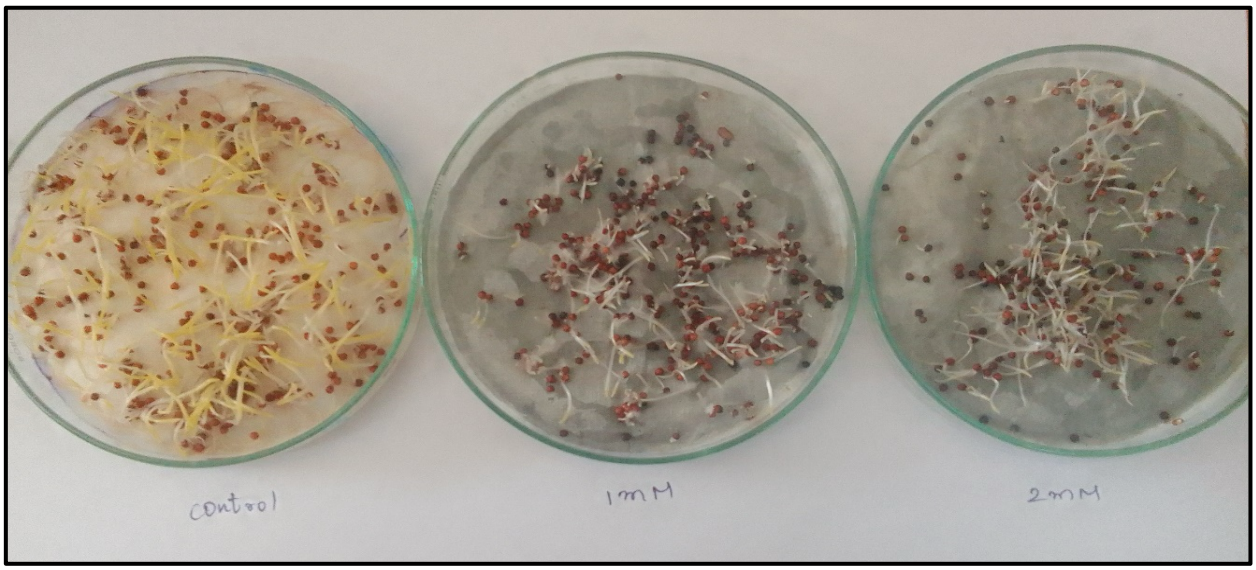


Figure:1: Germination setup of finger millet seeds using petriplate method

Finger millet seeds are treated with 1mM and 2mM sodium nitrate nanoparticles after overnight soaking with water and the effect on Germination is monitored. Both 1mM and 2mM NPs promoted germination but the shoot growth is found to be higher with 2mM NPs.

Figure:2 Root and shoot length of finger millet seeds treated with nitrate nanoparticles

Measurement No.	Control (Root)	Control (Shoot)	1 mM (Root)	1 mM (Shoot)	2 mM (Root)	2 mM (Shoot)
1	1.8	1.2	0.9	0.9	1.4	1.2
2	3.3	2.2	2.0	1.3	2.2	1.2
3	5.0	2.6	1.4	1.2	1.9	1.3
4	6.0	2.0	0.5	1.5	0.9	0.9
5	3.2	1.3	0.5	1.2	1.1	1.1
6	4.0	2.0	0.3	1.3	1.6	1.2
7	5.2	2.0	1.2	1.2	0.5	1.0
8	4.0	1.4	1.5	0.9	1.0	1.2
9	1.4	1.2	0.5	0.8	1.3	1.0
10	5.0	1.2	0.9	1.0	1.4	0.9
11	1.4	1.0	1.2	0.4	2.0	0.8
12	0.5	1.3	1.4	1.0	1.2	0.7
13	2.3	0.8	0.3	0.6	1.5	0.7
14	1.0	0.1	1.0	0.9	0.5	1.1
15	3.4	1.8	0.6	0.9	1.4	0.3

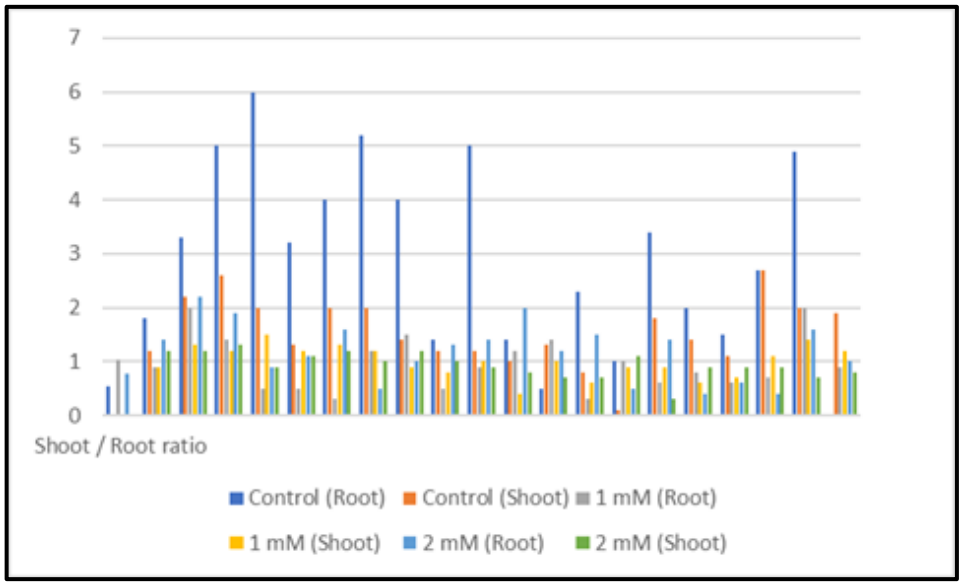
16	2.0	1.4	0.8	0.6	0.4	0.9
17	1.5	1.1	0.6	0.7	0.6	0.9
18	2.7	2.7	0.7	1.1	0.4	0.9
19	4.9	2.0	2.0	1.4	1.6	0.7
20	0.0	1.9	0.9	1.2	1.0	0.8
Average length in cm	**2.93**	**1.56**	**0.96**	**1.005**	**1.20**	**0.94**
Shoot / Root ratio	**0.53**		**1.04**		**0.78**	

Table:1 Length in cm, Average length and Shoot/root ratio of finger millet seeds treated with Nitrate nanoparticles

From table 1 mean values of shoot length and root length are measured in control, 1mM NPs and 2mM NP treated groups by selecting the seeds randomly. Shoot/root ratio is calculated for the same and highest value is encountered in 1mMNP treated group. Carbohydrate concentration in germinated seeds is estimated by anthrone method and highest value is recorded in 2mM NP treated group with a value of around 0.24mg/0.5g/ml.

Total reducing sugar content present in tested groups and control is estimated using DNS method and higher value recorded in 2mM NP treated group of 1.82mg/0.5g/ml. Total carbohydrate content is lesser in 1mM NPs treated groups and control due to high-rate metabolism and protein catabolism contributing to glucogenic compounds. Lipid mobilisation is higher in 2mM treated groups with negative values compared to 1mM treated group and control due to may be non-availability of carbohydrates

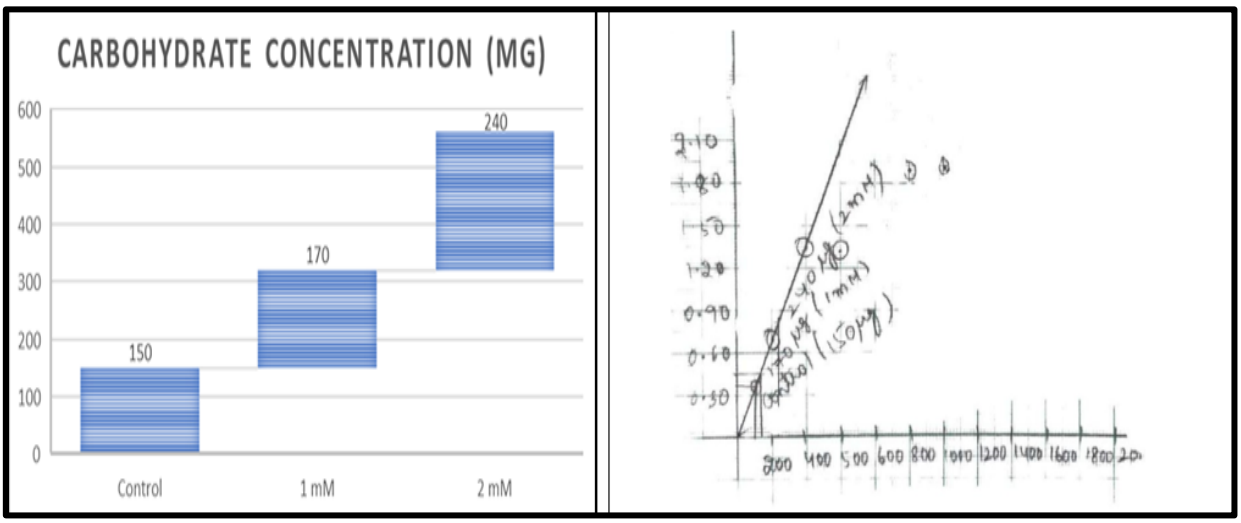
Protein concentration is higher in 2mMNP treated group compared with 1mM and control but protein conversion to glucogenic compounds is less due to low metabolic rate and as carbohydrates are the primary source for energy generation (unutilised in 2mM treated group).



Graph:1 Bar graph with defined shoot/root ratio, length of shoot and root in cm treated with 1mM, 2mM nitrate nanoparticles in finger millet.

Treatment	OD Value	Carbohydrate Concentration (mg)
Control	0.38	0.15 mg
1 mM	0.41	0.17 mg
2 mM	0.86	0.24 mg

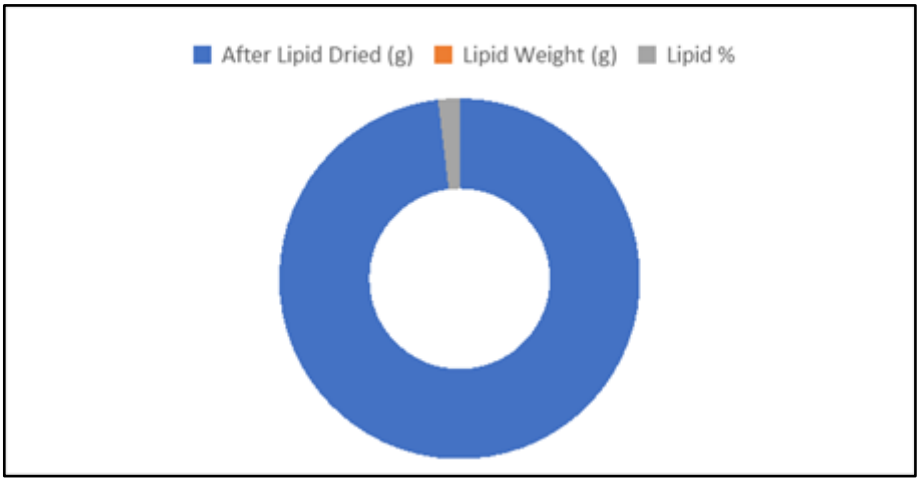
Table:2 Carbohydrate Concentration in mg of germinated finger millet seeds treated with 1mM and 2mM nitrate nanoparticles.



Graph:2 Bar graph of carbohydrate concentration in mg in 1mM and 2mM NP treated groups

Treatment	Empty Dish (g)	After Lipid Dried (g)	Lipid Weight (g)	Lipid %
Control	41.284	41.292	0.008	0.8%
1 mM	45.791	45.794	0.003	0.3%
2 mM	40.864	40.043	–0.821	–82.1%

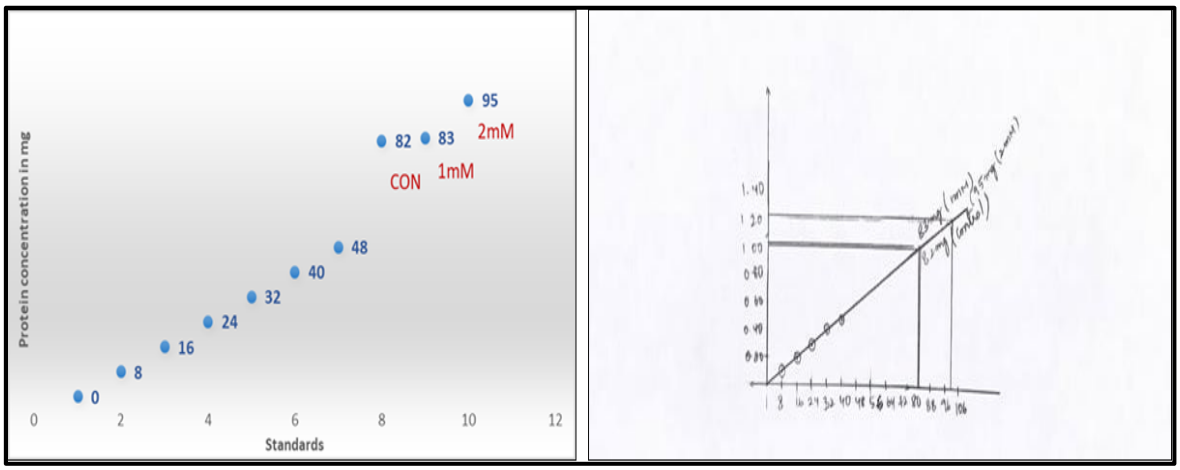
Table:3: Lipid content in percentage in finger millet seeds after germination treated with 1mM and 2mM concentrations of sodium nitrate nanoparticles



Graph:3 Graph showing Dried lipid content (g), Lipid weight (g) and Lipid % in finger millet seeds after germination

Tube No.	Sample Type	OD at 540 nm	Concentration
Blank	Blank	0.0987	0.00
1	Standard 1	0.0920	8mg
2	Standard 2	0.1786	16mg
3	Standard 3	0.2818	24mg
4	Standard 4	0.3914	32mg
5	Standard 5	0.5104	40mg
6	Standard 6	1.3197	48mg
7	Control Sample	1.1869	82 mg
8	1 mM Treated	1.2267	83mg
9	2 mM Treated	1.2267	95mg

Table:4: Protein concentration in mg in finger millet seeds treated with 1mM and 2mM NPs

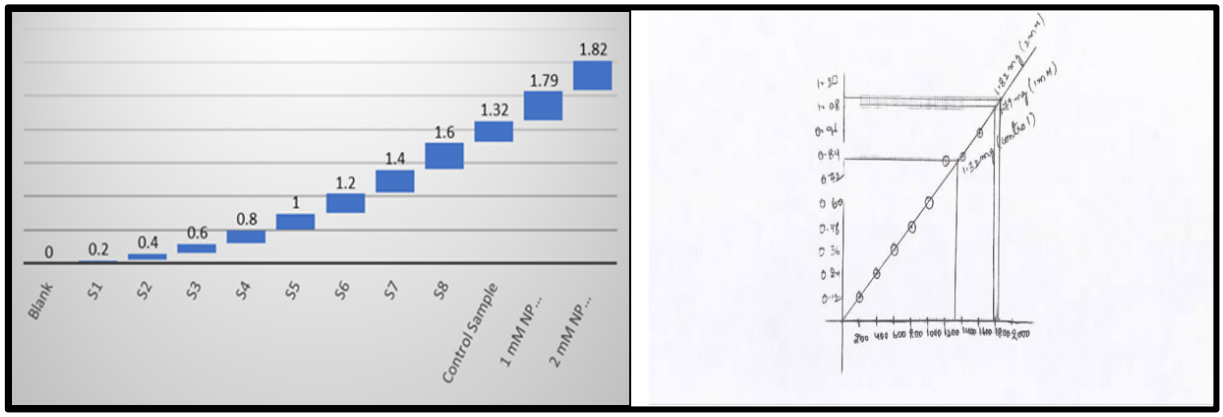


Graph:4 Graph showing protein concentration of finger millet seeds in mg treated with 1mM and 2mM nitrate nano particles.

Protein concentration value is recorded high in 2mM NPs treated group (95mg/0.5g/ml) where as in 1mM NP treated group and control it is of about 83mg/0.5g/ml and 82mg/1ml.

Tube No.	**Glucose Solution (ml)**	**Volume Made to 2 ml**	**OD Value (540 nm)**	**Concentration**
Blank	0.0	2.0	0.00	0.00
S1	0.2	2.0	0.12	200 µg
S2	0.4	2.0	0.24	400 µg
S3	0.6	2.0	0.36	600 µg
S4	0.8	2.0	0.48	800 µg
S5	1.0	2.0	0.60	1000 µg
S6	1.2	2.0	0.72	1200 µg
S7	1.4	2.0	0.84	1400 µg
S8	1.6	2.0	0.96	1600 µg
S9	Control	—	0.83	1.32 mg
S10	1mM NPs	—	1.09	1.79mg
S11	2mM NPs	—	1.11	1.82mg

Table: 5 Concentration of reducing sugars present in germinated seeds of finger millet treated by 1mM and 2mM nitrate NPs



Graph:5 Reducing sugars concentration in mg in germinated finger millet seeds treated with 1mM and 2mM sodium nitrate nanoparticles.

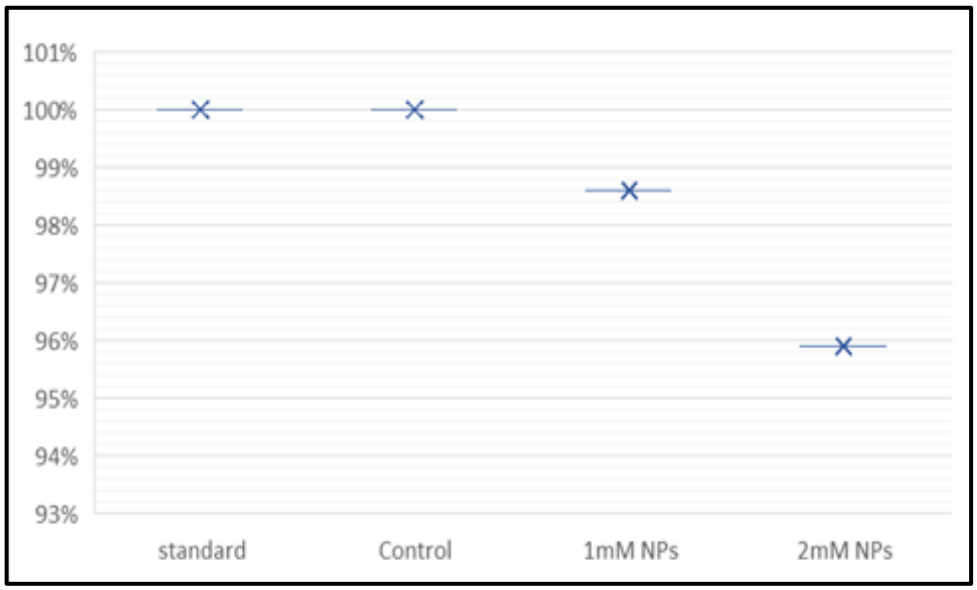
Antioxidant scavenging capacity was determined by using H2O2 method and the values are recorded more less similar in all groups when compared to standard but 100% scavenging capacity is recorded with control followed by 1mMNP treated group and 2mMNP treated group.

The antioxidant capacity was calculated according to the formula mentioned below

$$\% \text{ of Scavenging of } H_2O_2 = A_0\text{-}A_1/A_0 \text{ X}100$$

Tube	**Absorbance**	**Antioxidant scavenging capacity**
Standard	10.0000	100%
Control	10.0000	100%
1mM NPs	0.1384	98.6%
2mM NPs	0.1406	95.9%

Table: 6: Antioxidant scavenging capacity of 1mMNP and 2mM NPs treated finger millet seeds with reference to Standard.



Graph: 6 Whisker Graph showing Antioxidant scavenging capacity of seedlings of finger millet treated with 1mM and 2mM nitrate NPs.

Catalase activity was recorded high in 2mM nanoparticle treated group with around 21.4% which indicates oxidative stress in this treated group compared to 1mM NP treated group and control with reference to standard. Whereas in control and 1mM treated groups there is no significant catalase activity and hence no metabolic stress.

Catalase activity of Test kU = 2.303/t log S'/S ------ (3.1)

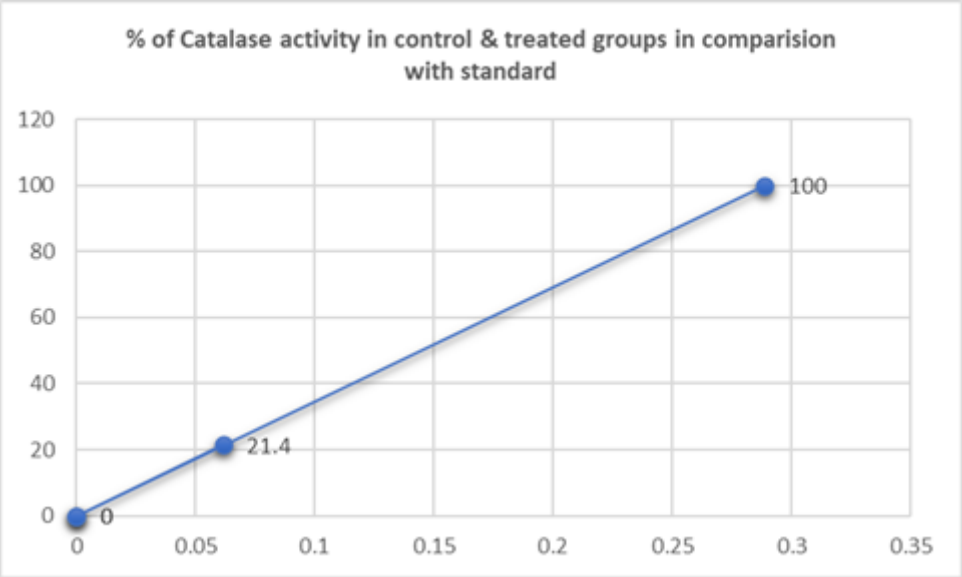
t = Time

S' : Absorbance of standard tube

S : Absorbance of Test tube

Sample	Absorbance at 440 nm	Catalase activity in % with reference to standard
Standard	0.2887	100%
Control	No Significant reading	No Free radicals produced
1mm	No Significant reading	No Free radicals produced
2mm	0.0618	21.4% (-2.03 acc. to formula 3.1)

Table:8 Comparision of catalase activity in % between treated groups and control with reference to standard



Graph:8 Graph showing % of Catalase activity in 2mM NPs treated group in comparision to standard

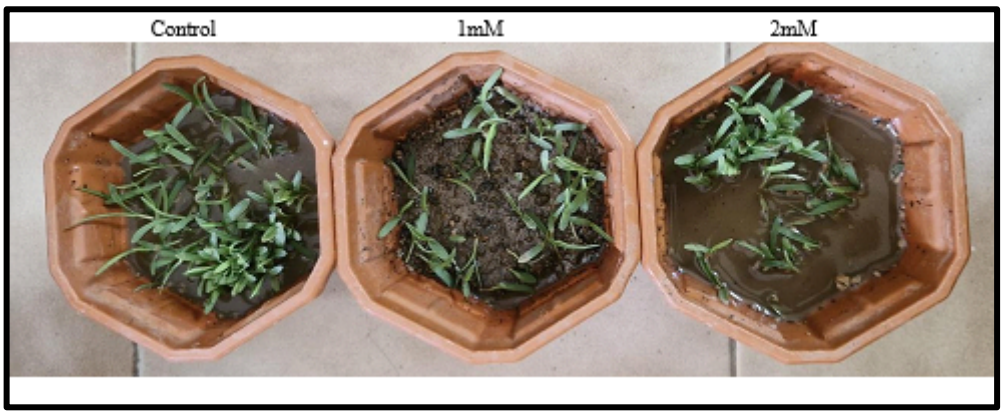
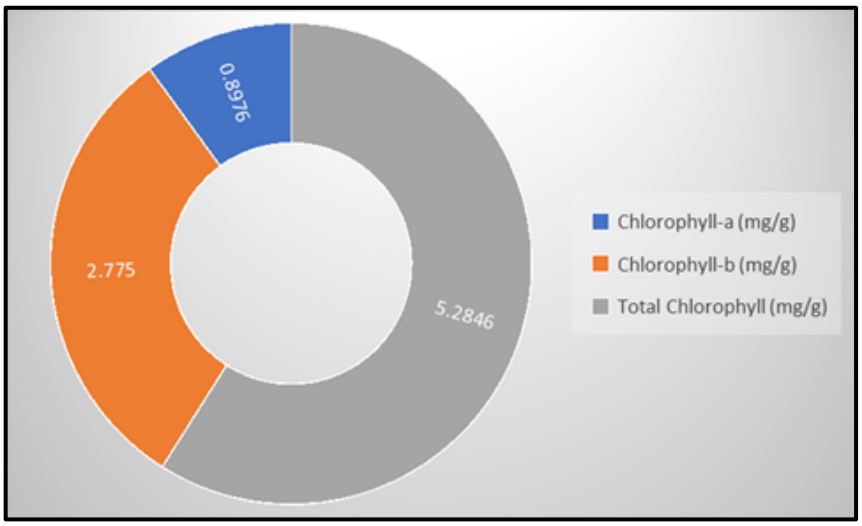


Figure: 3 Germination setup for finger millet seeds by using pot method using germination mix

Figure.3 indicates the germination setup by pot method for control,1mMNP and 2mMNP treated groups with 1:1 ratio of soil to germination mix. Growth is better observed with control followed by 1mMNP treated group and moderate growth is observed with 2mMNP treated group.

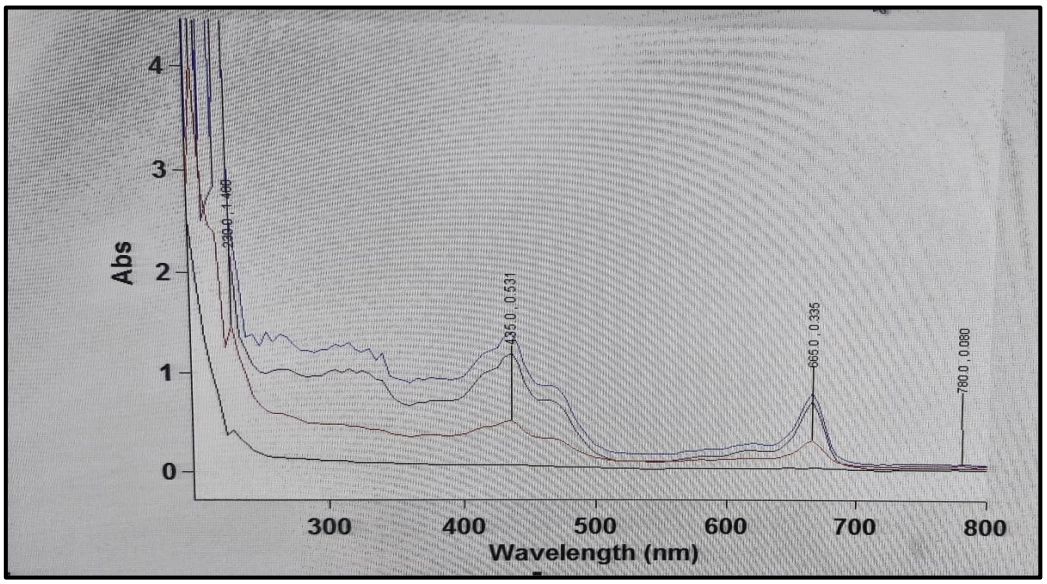
Treatment	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total Chlorophyll (mg/g)
Control	0.8976	2.7750	5.2846
1 mm	8.6353	6.7363	22.0666
2 mm	3.7264	3.0321	9.3794

Table:7: Chlorophyll a, Chl b and total content of chlorophyll present in germinated seedlings of finger millet in nanoparticle treated groups



Graph: 7: Graph with chla, chl b and total chlorophyll content present in germinated seedlings of finger millet seeds treated with NPs.

Chlorophyll content is measured in control,1mMNP and 2mMNP treated groups and highest values of Chl a, Chl b and total chlorophyll values are recorded with 1mMNP treated group (Chla: 8.6353, Chl b: 6.7363, Total chl: 22.0666) compared to control (Chla: 0.8976, Chl b: 2.7750, Total chl: 5.2846) and 2mMNP treated groups (Chla: 3.7264, Chl b: 3.0321, Total chl: 9.3794).



Scan:4: UV – visible scan of chlorophyll content in the wavelength range of 200 to 800nm (Chl a, Chlb and total chlorophyll). Characteristic peaks are observed at 435nm (Chlb) and 665nm (Chl a).

U.V Visible scan of chl a is carried at wavelength 663nm, chl b at 645nm and the total chlorophyll content is calculated by using the formulas

Chlorophyll a (mg/g) = (12.7*A663) - (2.59* A645)

Chlorophyll b (mg/g) = (22.9*A663) - (4.7* A645)

Total Chlorophyll (mg/g) =(8.2*A663) - (20.2* A645)

Discussion and Conclusion:

Finger millet (Eleusine coracana L.) is an allopolypoid with 36 chromosomes and one among its two diploid species Eleusine indica (AA) and Eleusine floccifolia or E. tristachya (BB) as its parental genome donors [4,5]. Finger millet is also useful in management of various physiological disorders such as vascular fragility, hypercholesterolemia, prevention of oxidation of lowdensity lipoproteins (LDLs) and improves gastrointestinal health[5,6,7,8,9]. The major components of the finger millet, polyphenol is not distributed in the seed fraction but stored in the top aleurone layer, testa and pericarp of the fruit and exists as both free soluble conjugates and insoluble bound forms[10]. Ferulic acid (64%–96%) and p-coumaric acid (50%–99%) are the major phenolic compounds found in the finger millets[11,12].

Studies on finger millet seeds treated with nanoparticle formulations proven to contain high content of protein, reducing sugars, carbohydrate rich and posess high free radical scavenging capacity compared to normal fertiliser treated crops due to high nitrate bioavailability.

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