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SYNTHESIS OF BIOLOGICAL NANOPARTICLES FROM PROTEUS AND THEIR EFFECT ON PLANTLET GROWTH

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Abstract

Proteus sps. such as *Proteus mirabilis* are used to synthesise AgNPS and MgNPs but till now there is no biological NPs with *Proteus vulgaris* and in this study we synthesised *Proteus vulgaris* derived FeNPs and studied for its effect on plantlet growth of pearl millet and germination efficacy. After germination the NPs treated groups and control are studied for their biochemical characteristics like total carbohydrate content, reducing sugar content, Lipid percentage, Protein content, Antioxidant scavenging capacity, Catalase activity and chlorophyll content present in the seedling. Pearl millet seeds treated with 2mM FeNPs + *Proteus* showed better growth and improvement inavailability of bionutrients like total carbohydrate content, reducing sugar content, Lipid percentage, Protein content, Antioxidant scavenging capacity, Catalase activity and chlorophyll content compared to other treated groups followed by 2mM FeNPs.

Key words: 2mM FeNPs; 2mM FeNPs + *Proteus*; Antioxidant scavenging capacity, Catalase activity, Total carbohydrate content, Reducing sugar content, Lipid percentage, Protein content.

Introduction:

Proteus bacteria can able to bind to metal ions (R. Augustine et al.(2020)) and till now *Proteus mirabilis* is used to synthesize biological nanoparticles by using green synthesis method. Studies of Iqteda.M et al.,(2024) proved synthesized Proteus MgNP's exhibited high anti microbial efficacy with most pathogenic MDR microbes like *Staphylococcus aureus* BTCB02 and *Salmonella typhi* BTCB06 through inhibiting the pathogen growth. According to the studies

of Yasr et al., (2022) synthesized *Proteus mirabilis* derived AgNP's showed to contain antioxidant potential and free radical scavenging capability by neutralizing oxygen/nitrogen radicals.

Microbial bioremediation through conversion of toxic selenite in to selenium NP's is gaining insight now and the studies of Liu et al., (2022) proved SeNPs derived from *Proteus mirabilis* YC801 has showed neural regeneration after spinal cord injury and inflammation there by known to possess neuroprotective properties. However the neural protection offered by SeNPs has to be elucidated with other CNS disorders.

The current study focus on synthesis of FeNPs derived from P.vulgaris and study their effect on the plantlet shoot /root growth and availability of bio-nutrients in the pearl millet treated with different concentrations of FeNPs and FeNPs derived from *Proteus vulgaris*.

Methodology:

1. Synthesis of FeNPs and Proteus derived FeNPs:

1mM and 2mM concentrations of NaCl and FeSo4 is used to synthesize FeNPs 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 5 days and characterized using UV- Visible spectrophotometer.

Synthesised FeNPs are used fo the synthesis of *Proteus* derived FeNPs. 50ml of 1mM FeNPs is aliquoted in to separate beaker. 5ml of Proteus culture is centrifuged at 2000rpm for 10-15 min and the supernetent is decanted carefully and added to 1mM and 2 mM FeNPs and allowed to stand at room temperature for 5 days and characterized using UV- Visible spectrophotometer.

2. Germination set up of Pearl millet seeds:

Petriplates and whatmann filter paper no:1 are sterilized using autoclave and experimental seeds are surface sterilized with 1% Sodium hypochlorite solution after over night soaking and are placed randomly in the petriplate placed with whatmann filter paper no:1 and sprinkled with 1mM and 2mM FeNPs and 1mM and 2mM *Proteus* derived FeNPs and observed for germination on the next day. The petriplates are sealed and kept in dark for 24 hrs.

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 FeNPs and 1mM and 2mM *Proteus* derived FeNPs and allowed for germination. 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:

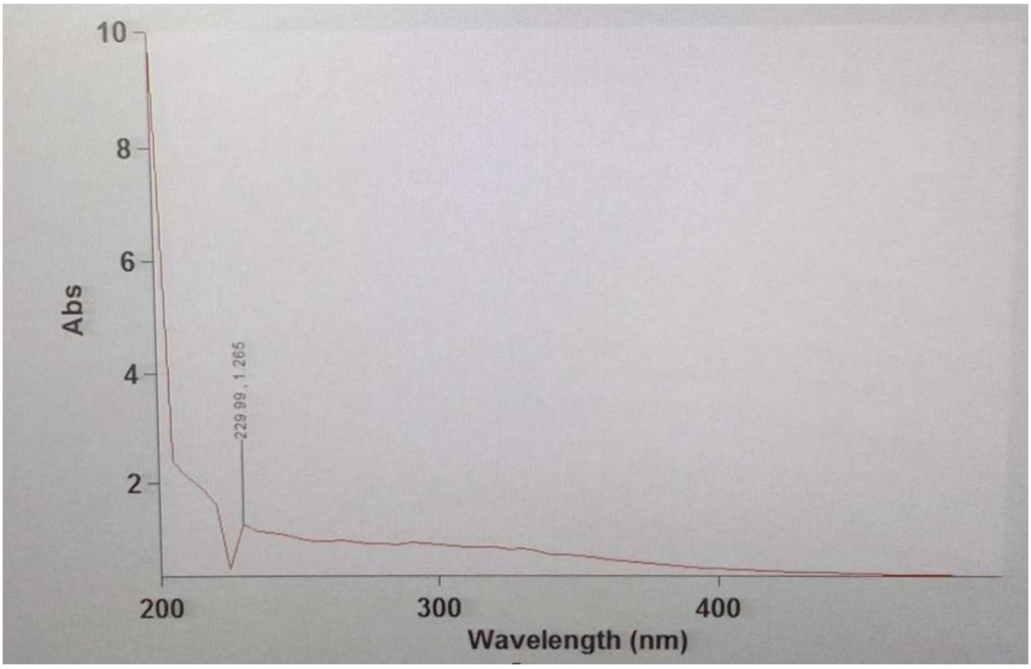


Figure:1 UV - Visible Scan of 1mM FeNPs in the wavelength range 200- 600nm.

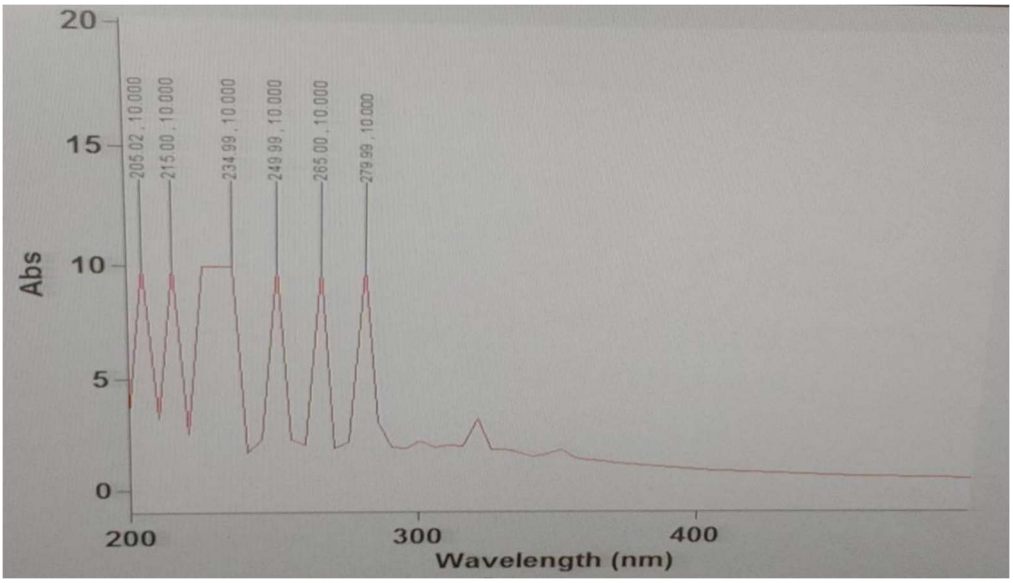


Figure:2 UV - Visible Scan of 2mM FeNPs in the wavelength range 200- 600nm

Synthesis of FeNPs and FeNPs derived from *Proteus vulgaris* is carried using NaCl and FeSO4 with 1mM and 2mM concentrations and the synthesized FeNPs is characterized using UV- Visible Spectrophotometer. Peaks are recorded in the range of 229 nm and 250-400nm with 1mM and 2mM FeNPs and 424 nm with 1mM FeNPs derived from Proteus. Peaks of 2mM FeNPs derived

from Proteus showed shift in absorbance to higher wavelengths (Hyperchromic shift) usually caused due to shift in double bonds and Auxochrome effect.

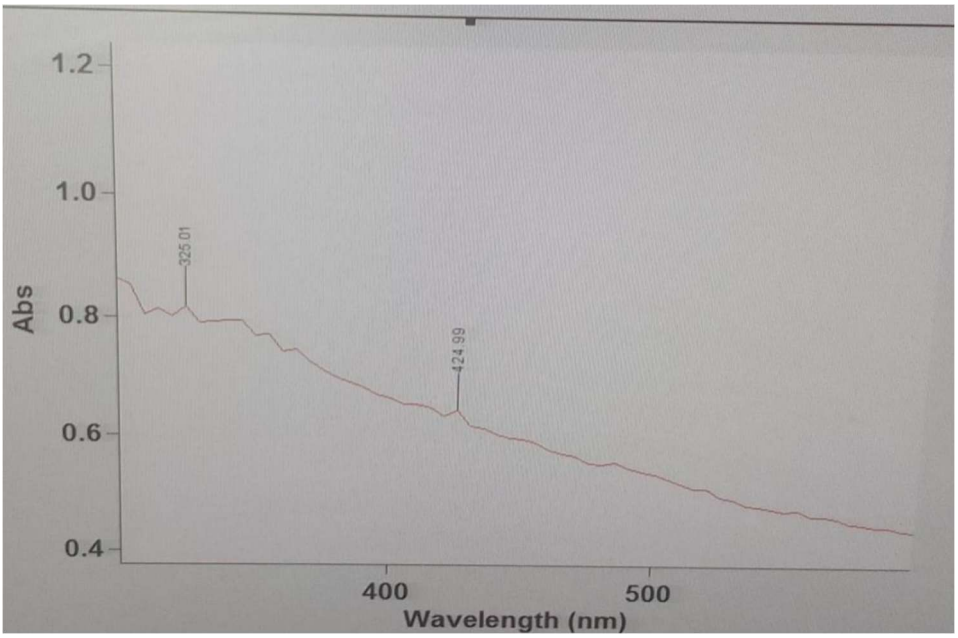


Figure:3 UV - Visible Scan of 1mM *Proteus* FeNPs in the wavelength range 200- 600nm

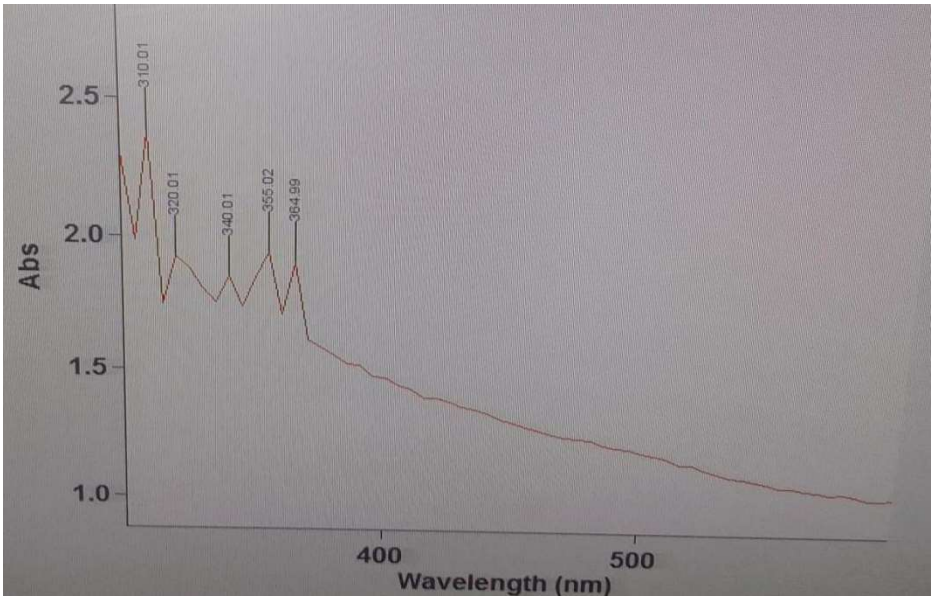


Figure:3 UV - Visible Scan of 2mM *Proteus* FeNPs in the wavelength range 200- 600nm.

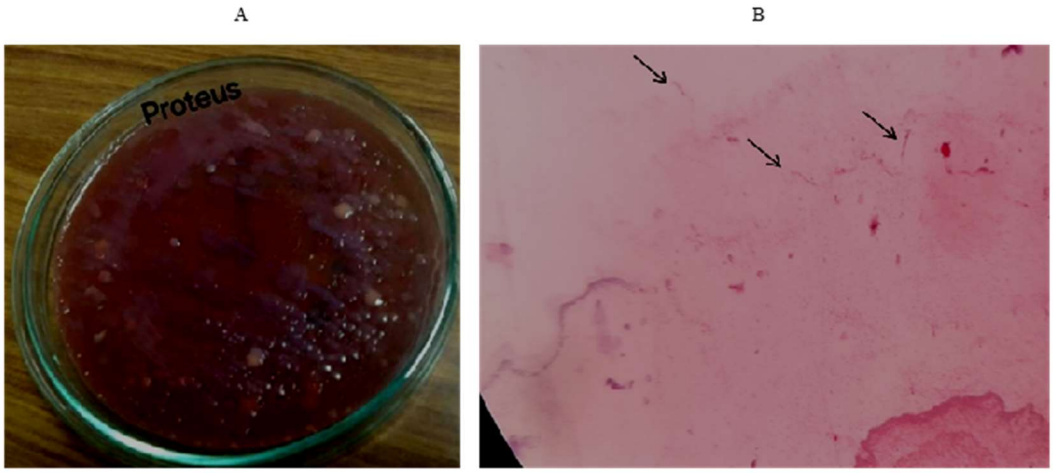


Figure: 4 Isolation and Characterization of *Proteus vulgaris* from urine sample: (A) Growth of *Proteus vulgaris* on selective media Macconkey agar w/o CV, NaCl w/ 0.5% Sodium Taurocholate. (B) Gram staining results of the bacteria grown on Macconkey agar plate.

Urine sample is used to isolate the *Proteus vulgaris* for the synthesis and pure culture is maintained. The colonies formed on the macconkey agar plate is studied for their morphological characteristics and identified as either gram positive or gram negative using gram staining Procedure. The bacteria is identified as *Proteus vulgaris* a gram negative rod with peritrichous flagella. Selective media, Macconkey agar w/o CV, NaCl w/ 0.5% Sodium Taurocholate is used for isolation of *Proteus vulgaris*.

Figure: 5: Germination setup for pearl millet using Petri plate method. (A) Plates showing Germinated pearl millet seedlings. (B) Shoot and root characterization in pearl millet seedlings.

Petri plate method is used for studying the germination of Pearl millet seeds and seeds treated with 2mM FeNPs derived from *Proteus* recorded with high shoot/root length than 1mM FeNPs derived from Proteus, 2mM FeNPs and Control. Germination efficacy is also proven to be more with 1mM & 2mM FeNPs derived from *Proteus* and proven to be more or less similar to control (1mM) and

greater than control (2mM) than compared with 1mM &2mM FeNPs. The average germination (%) in case of 1Mm NP + *Proteus* is found to be 8.89 % and with 2Mm NP+ *Proteus* is found to be 38.09 %. In control the average germination percentage is found to be 11.89 % and 2mM FeNPs recorded with high average germination(%) compared to control with 14.99 %.

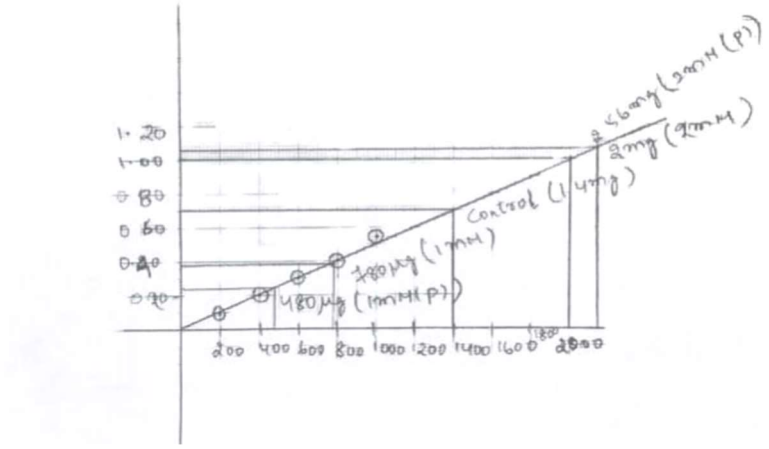
S.No	Treatment Group	Avg. Germination (%)
1.	Control	11.89
2.	1Mm NP	1.53
3.	2Mm NP	14.99
4.	1Mm NP + *Proteus*	8.89
5.	2Mm NP+ *Proteus*	38.09

Table 1 : Average Germination Percentage (%) in pearl millet seedlings treated with FeNPs and FeNPs derived from *Proteus*.(1Mm NP + *Proteus* = FeNPs derived from *Proteus* synthesized using Proteus at 1mM concentration of FeCl3 NPs, 2Mm NP + *Proteus* = FeNPs derived from *Proteus* synthesized using *Proteus* at 2mM concentration of FeCl3 NPs).

Average Germination Percentage (%): Shoot/Root ÷ No.of samples x 100

S.No	Treatment Group	Absorbance (OD at 720 nm)	Carbohydrate Concentration (mg)
1.	Control	0.87	1.25
2.	1 mM NP	0.38	0.55
3.	2 mM NP	1.00	1.45
4.	1 mM NP + *Proteus*	0.24	0.36
5.	2 mM NP + *Proteus*	1.06	1.53

Table 2: Carbohydrate Estimation in germinated pearl millet seedlings treated with 1mM & 2mM FeNPs and FeNPs derived from *Proteus* of 1mM & 2mM by Anthrone method.

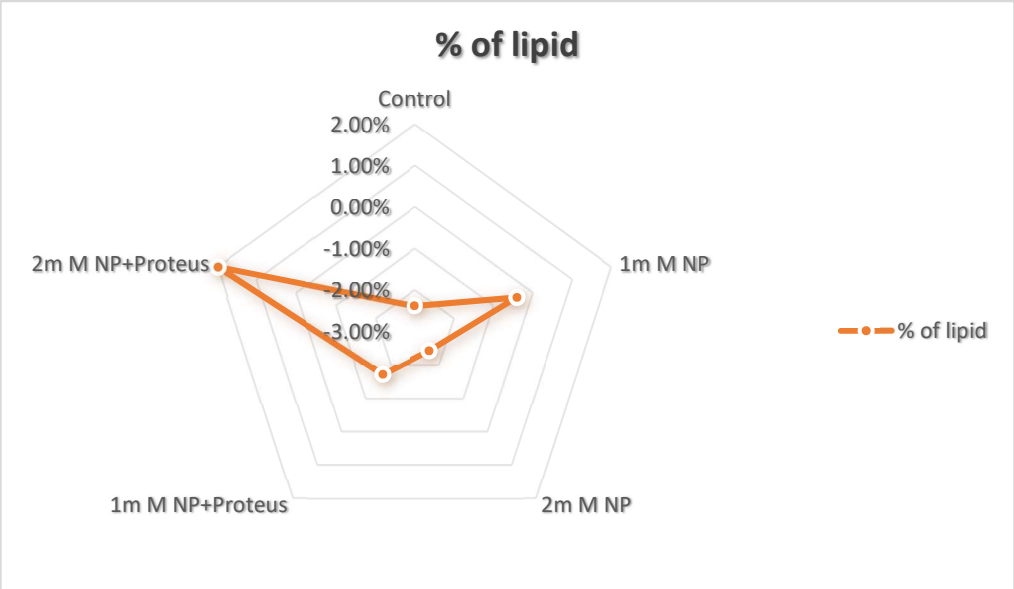


Graph: 1 Graph showing the concentration of total carbohydrate content present in pearl millet seedlings treated with1mM & 2mM FeNPs and FeNPs derived from *Proteus* synthesised using 1mM & 2mM FeCl3 NPs.

Total carbohydrate content is estimated using anthrone method and the total carbohydrate content in control treated group is found to be around 1.4 mg, where as in case of 1mM and 1mM FeCl3+ *Proteus* NP's treated groups it is found to be 780µg and 480µg respectively. 2mM FeNP's and 2mM FeCl3 + *Proteus* NP's treated seeds proven to contain 2mg and 2.56 mg of total carbohydrate content collectively after germination into seedlings. Lipid % is found to be negative with all the treated groups except in case of 2mM NP's + *Proteus* may be due to lipid mobilization by Glyoxalate pathway in other treated groups during germination .

S.No	Treatment Group	Before (g)	After(g)	Lipid content (g)	% of lipid
1.	Control	45.424	45.400	(-) 0.024	-2.4%
2.	1m M NP	43.045	43.041	(-) 0.004	-0.4%
3.	2m M NP	44.016	43.992	(-) 0.024	-2.4%
4.	1m M +*Proteus*	41.487	41.470	(-) 0.017	-1.7%
5.	2m M NP+ *Proteus*	42.460	42.480	0.020	+2.0%

Table 3 : Lipid Content in Germinated pearl millet seedlings treated with 1mM & 2mM FeNPs and with *Proteus* derived NPs synthesised using 1mM & 2mM FeCl3 NPs.



Graph:2 Radar Graph showing Lipid percentage in Germinated pearl millet seedlings treated with FeNPs and *Proteus* Biological NPs;1mM NP = 1mM FeNP, 2mM NP = 2mM FeNP,1mM+ *Proteus* = 1mM FeNPs *Proteus*, 2mM+ *Proteus* = 2mM FeNPs derived from *Proteus*.

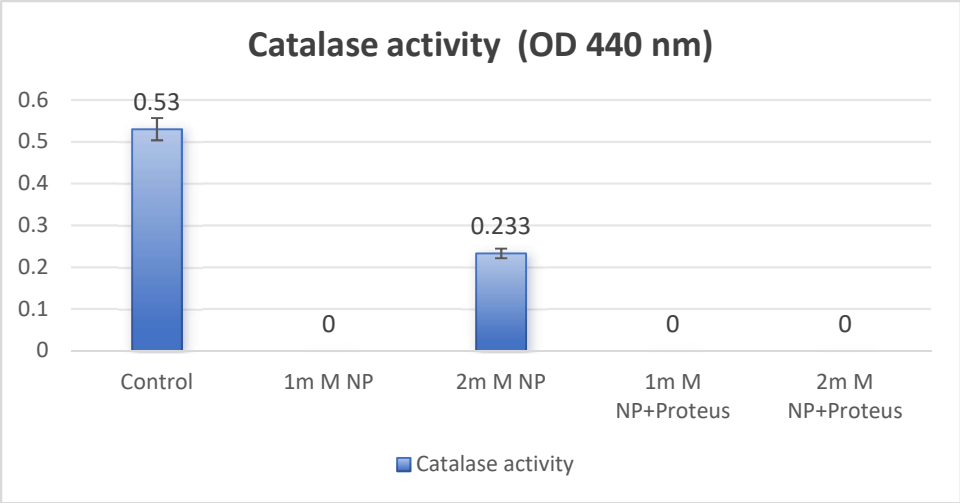
S.No	Treatment Group	Catalase activity (OD 440 nm)
1.	Control	0.530
2.	1m M NP	0.000
3.	2m M NP	0.233
4.	1m M+ *Proteus*	0.000
5.	2m M NP+*Proteus*	0.000

Table 4: Assessment of Catalase Activity of germinated pearl millet seeds in control and treated groups

Catalase activity of Test kU = 2.303/t log S'/S

t = Time

S' : Absorbance of standard tube
S : Absorbance of Test tube



Graph 3 : Bar Graph of Catalase Activity (OD at 440 nm) in control and treated groups.

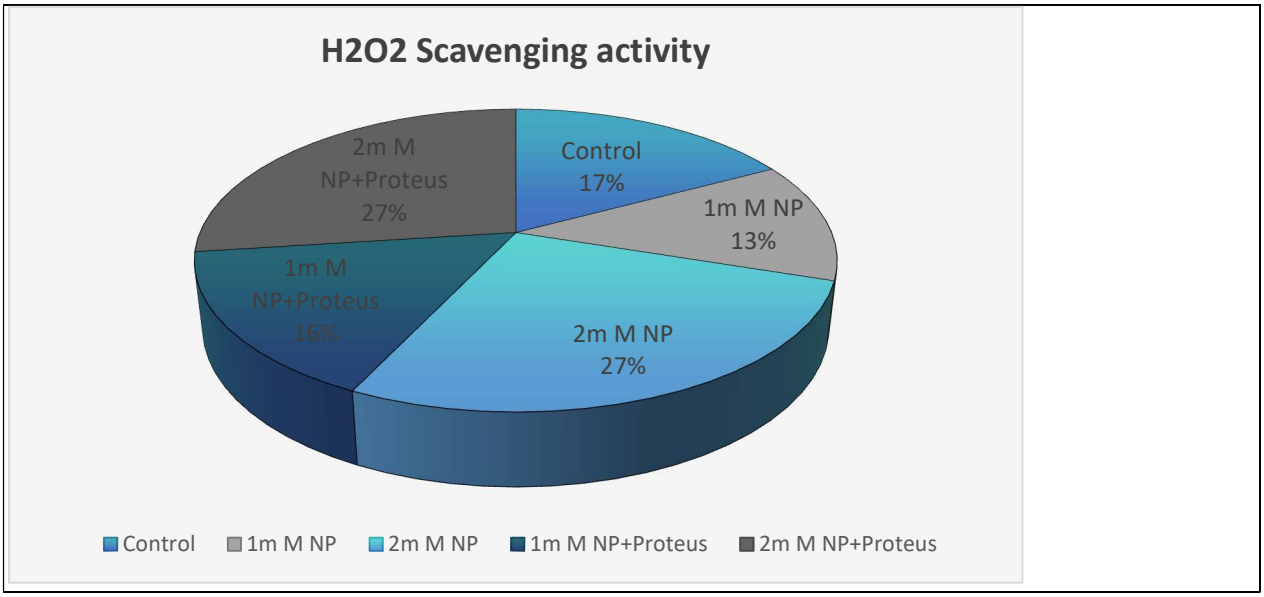
Germinated seedlings are assayed for the catalase activity to calculate the stress and it is almost zero in case of 1mM FeNPs, 1mM FeNPs + *Proteus* and 2mM FeNPs + *Proteus* and the catalase activity was found to be high in control and 2mM FeNPs indicates high free radical production in the control and 2mM FeNPs treated groups.

S.No	**Treatment Group**	**H2O2 Scavenging (%)**
1.	Control	63.6%
2.	1m M NP	47.5%
3.	2m M NP	100%
4.	1m M NP+ *Proteus*	58.7%
5.	2m M NP+ *Proteus*	100%

Table 5 : Antioxidant Assay in control seeds and seeds treated with NP's

Antioxidant assay was carried using H2O2 scavenging method and from table 5 and Graph 4 the scavenging capacity is found to be 100% with 2mM FeNP's and 2mM FeNP's + *Proteus* treated groups followed by control, 1mM FeNP's + *Proteus* and 1mM NP's treated groups. Total protein content present in control,1mM NP's and 2mM NP's is found to be 36mg/ml, 20mg/ml and 15mg/ml per 0.1g of germinated seedling sample used. Where as in 1mM FeNP+*Proteus* and 2mM FeNP+ *Proteus* treated seeds it is found to be about 24mg/ml and 17mg/ml per 0.1g of germinated seedling sample in weight.

% of Scavenging of H_2O_2 = A_0-A_1/A_0 X100



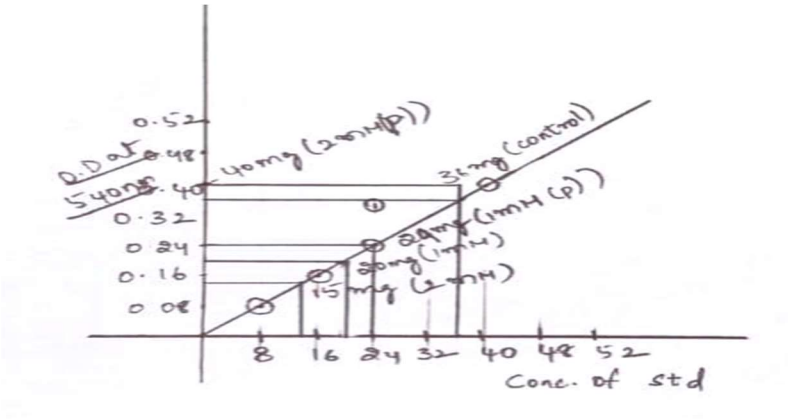
Graph 4 : H_2O_2 Scavenging Activity at 230 nm (Antioxidant Assay) of germinated control seeds and FeNPs treated germinated seedlings.

S.No	Treatment Group	OD (540 nm)	Protein concentration (mg/ml)
1.	Control	0.36	36 mg/ml
2.	1m M NP	0.20	20 mg/ml
3.	2m M NP	0.15	15 mg/ml
4.	1m M NP+*Proteus*	0.24	24 mg/ml
5.	2m M NP+*Proteus*	0.17	17 mg/ml

Table 6 : Protein estimation using biuret method in control and FeNPs treated germinated seedlings.

Total reducing sugar content present in control, 1m M NP's and 2m M NP's treated groups is recorded to be 1.3mg ,1.02mg and 900 µg/0.1g of germinated seedlings and 1mM FeNP+*Proteus* and 2mM FeNP+*Proteus* treated groups is shown to contain about 1.32mg and 1.18mg of reducing sugar content/0.1g of germinated seedling sample. Total reducing sugar

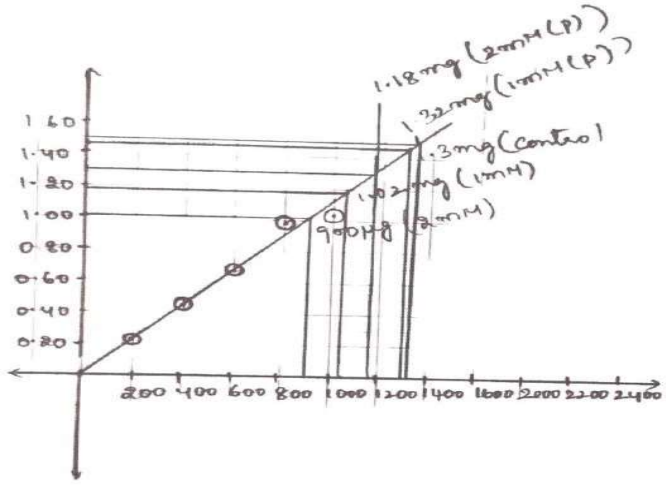
content present in germinated seedlings is found to be less than total carbohydrate content in all treated groups.



Graph:5: Graph showing amount of Protein in mg present in pearl millet seedlings treated using distilled water (Control) and 4 different FeNPs Groups. (1mM FeCl3,2mM FeCl3,1mM FeCl3+ *Proteus* and 2mM FeCl3+ *Proteus*).

S.No	**Treatment Group**	**OD at 540 nm**	**Reducing sugar content**
1.	Control	1.44	1.3 mg
2.	1 mM NP	1.18	1.02 mg
3.	2 mM NP	1.09	900 µg
4.	1 mM NP + *Proteus*	1.48	1.32 mg
5.	2 mM NP + *Proteus*	1.28	1.18 mg

Table 7 : Reducing sugar content (mg) in Germinated seedlings of control and FeNPs treated groups.



Graph:6 : Amount of reducing sugar in mg present in germinated seedlings of pearl millet in control and FeNPs treated groups.

Germination of pearl millet seeds are carried out using pot method to estimate the total chlorophyll content in ll treated groups. Chlorophyll a content is found to be higher in all nanoparticle treated groups except control with chlorophyll b as the major Chlorophyll.

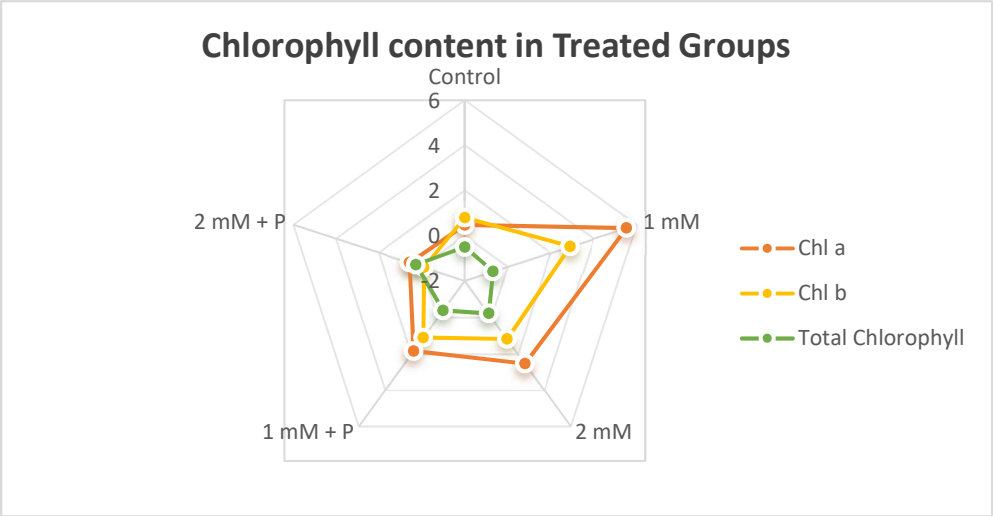
S.NO	reatment group	Visual Germination Result
1.	Control	Dense and healthy growth
2.	1m M NP	Low to moderate germination
3.	2m M NP	Dense and high germination
4.	1m M NP+*Proteus*	Dense and high germination
5.	2m M NP+*Proteus*	Very poor/ no germination

Table 8: Germination efficacy of control and FeNPs treated seeds in pots using Germination mixture.

Figure: 6 Germination setup for Pearl millet using Pot technique

S.No	Sample	A645	A663	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Total Chlorophyll (mg/L)
1.	Control	0.0439	0.0456	0.4654	0.7909	(-) 0.5128
2.	1 mM	0.2260	0.4819	5.5344	2.9104	(-) 0.6814
3.	2 mM	0.0977	0.2208	2.5511	1.1995	(-) 0.1922
4.	1 mM + P	0.0830	0.1637	1.8640	1.1313	(-) 0.3591
5.	2 mM + P	0.0053	0.0464	0.5755	(-) 0.0967	0.2734

Table 9 : Chlorophyll content in plantlets treated with FeNPs nanoparticles and *Proteus* derived FeNPs.



Graph 7: Chlorophyll content present in Leaves of Pearl millet in control and FeNPs treated groups

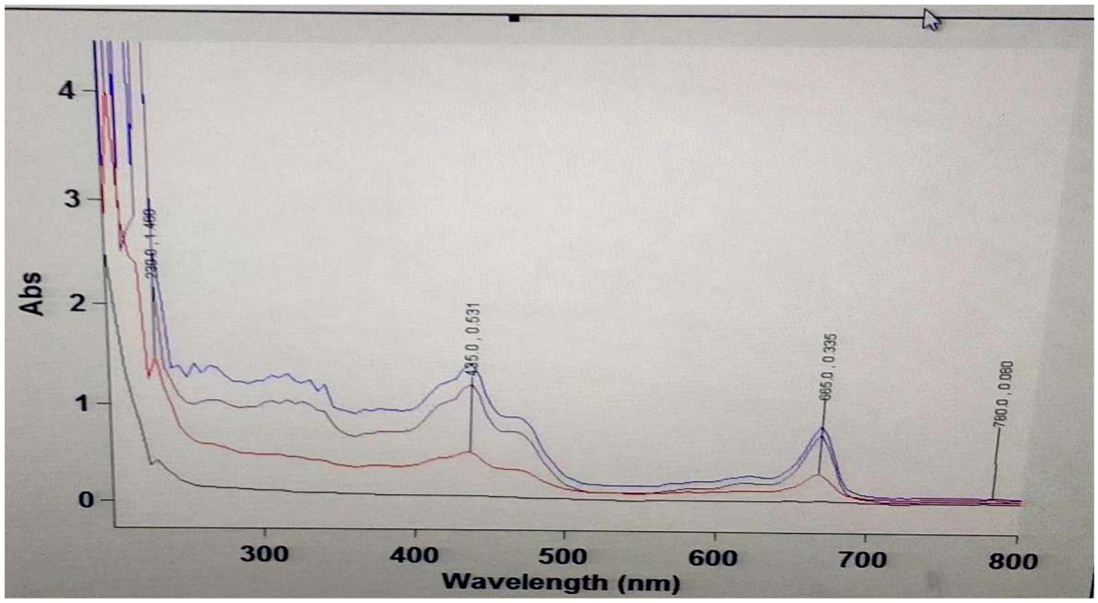


Figure:7 UV - Visible scan of Chlorophyll a and Chlorophyll b in samples treated with distilled water (control) and 2mM FeNPs and 1mM FeNPs+ *Proteus*

Chlorophyll a (mg/g) = (12.7*A663) - (2.59* A645)
Chlorophyll b (mg/g) = (22.9*A663) - (4.7* A663)
Total Chlorophyll (mg/g) =(8.2*A663) - (20.2* A645)

Discussion and Conclusion:

Urinary tract infections are mainly caused by *P. mirabilis* through catheter occlusion because of swarming motility associated with the pathogens. Swarming motility is is seen in many pathogenic microbes to exclude antimicrobial agents and from the studies of Mahdi et al., (2024), artificial zinc oxide nanoparticles (ZnO NPs) made from *E. Faecium* shown to possess anti microbial property by effecting biofilm formation and reduced swarming motility through down regulation of rsbA gene expression in P.mirabilis.

Zinc-oxide nanoparticles (ZnO NPs) synthesised by *Cycas circinalis* showed antimicrobial properties against systemic infections caused by *Proteus mirabilis* through compromising the membrane integrity in *Proteus* (Elekhnawy et al., (2023)).Synthesized copper nanoparticles using a carbohydrate-based bioflocculant derived from *Proteus mirabilis* AB 932526.1. can be used for waste water treatment and as an anti microbial agent. From the studies of Nkanyiso C. Nkosi et al., (2025) synthesized copper nanoparticles can remove dyes and nutrient effluents like saffranin and bio minerals like Nitrogen, sulfate, Phosphate and total nitrate content present in domestic waste water. Finally microbial derived nanoparticles showed high anti microbial potential against most of the pathogenic microbes and in current study synthesized FeNPs derived from *Proteus*

vulgaris showed increased accumulation of bionutrients like carbohydrates and proteins and the Fe nanoparticle treated seedlings exhibited high antioxidant potential and free radical activity along with high content of chlorophyll a in leaves.

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