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EFFECT OF MAGNESIUM FERRITE ($MGFE_2O4$) NANOPARTICLES ON THE GROWTH AND DEVELOPMENT OF *SPINACIA OLERACEA* (SPINACH PLANTS)

Palash. Manoj Thakre[1a], Shardul S. Wagh[1b], Bhuwaneshawari. A. Mehere[1a*], Manisha M. Jiwatode[2], Jyotsna V. Khobragade[2]

[1a]Department of Biochemistry, Dr. Ambedkar College, Deekshabhoomi, Nagpur, 440010 (India)
palashthakre21@gmail.com , bhumimehere@gmail.com

[1b]Department of Biochemistry, Kamla Nehru Mahavidyalaya, Maharashtra, Nagpur, 440024 (India)
shardulwagh@gmail.com

[2]Department of Chemistry, Guru Nanak College of Science, Ballarpur 442701(India).
manisha.jiwatode186@gmail.com ,
jdr2105@gmail.com

Corresponding Author: palashthakre21@gmail.com

Abstract: Nutrient depletion caused by heavy rainfall and excessive irrigation significantly affects soil fertility and plant productivity. The present study investigated the impact of nutrient loss induced by soil washing on the growth and biochemical performance of *Spinacia oleracea* (spinach plant) and evaluated the potential of magnesium ferrite nanoparticles to mitigate nutrient deficiency stress. Normal soil (C1) and washed soil (C2) were used as control groups, while washed soil supplemented with magnesium ferrite nanoparticles at 10, 20, 40, and 80 mg per 100 g soil constituted the treatment groups (E1–E4). Plant growth parameters, including germination rate, vigour index, and fresh biomass, along with biochemical parameters such as protein, reducing sugar, chlorophyll, and proline contents, were assessed at 15, 30, and 60 days after germination. Plants grown in washed soil exhibited reduced biomass, chlorophyll, protein, and reducing sugar levels, accompanied by elevated proline accumulation, indicating nutrient-deficiency induced stress. Supplementation with magnesium ferrite nanoparticles significantly improved growth and biochemical parameters in a concentration-dependent manner and reduced proline accumulation compared to washed-soil controls. The findings demonstrate that magnesium ferrite nanoparticles can partially restore plant growth and metabolic performance under nutrient depleted soil conditions, highlighting their potential as a nano-amendment for improving crop productivity in leached soils.

Keywords: Magnesium Ferrite, biochemical parameters, soil fertility, nutrient depletion.

1. Introduction

Heavy rainfall and excessive irrigation are major environmental factors, responsible for nutrient depletion in agricultural soils which primarily cause the leaching of essential mineral nutrients from the topsoil [1]. Most plant nutrients, including calcium, magnesium, iron, and other micronutrients, predominantly present in the soil in their ionic forms, which makes them highly liable to washing away during continuous water percolation [2]. Repetition of, soils exposed to washing led to the loss of their mineral content which creates the nutrient-deficient conditions that affect the plant growth and development [3]. Plants grown under the nutrient

depleted condition exhibit reduced in photosynthetic efficiency and affect the biochemical parameters such as protein, carbohydrate, and chlorophyll [4].

Under nutrient leaching conditions, conventional soluble fertilizers are also prone to rapid loss from soil, which significantly decreases their utilization efficacy by plants and contributing to environmental imbalance and indirectly causes the plant nutrient quality [5]. This requires the development of alternative nutrient strategies that will not be washed away easily and remain available in the rhizosphere, and provide sustained nutrient release over time [6]. In this situation, nanotechnology-based approaches have emerged as promising solutions for improving agriculture problem [7].

Metal nanoparticles have gained attention due to their high surface area, reactivity, and ability to interact effectively with soil particles and plant root systems [8]. Unlike free metal ions, nanoparticles exist in a particulate form that decreases their instant leaching from soil, thus improving their persistence and bioavailability [9]. Nanoparticles can function as slow release nutrient sources, supplying essential elements to plants in a controlled and sustained manner hence there is a need of searching their application for solving environmental problem [10].

For evaluating whether the synthesized particle can be effective on plant growth and development there is a need of selection of specific and divalent metal which persist its vital role in living system and that divalent metal is magnesium [11]. Magnetic nanoparticles such as magnesium ferrite (MgFe2O4) includes additional advantages due to their strong interaction with soil, which may enhance their holding within the soil matrix under gravitational forces and natural magnetic interactions [12]. Magnesium ferrite nanoparticles can serve as a dual source of magnesium and iron; both are essential for synthesis of chlorophyll [13]. The regular release of these metal nutrients from magnesium ferrite nanoparticles may help less nutrient stress and improve plant growth and biochemical performance in soils targeted to nutrient loss due to washing or heavy rainfall [14]. In this work the effect of magnesium ferrite nanoparticle was evaluated on spinach plant. For the experiment the wash soil was prepared in laboratory and experiment was conducted successfully first time by giving the nanoparticle treatment to nutrient depleted soil. The different growth parameters and biochemical analysis was done by following respective protocol.

2. Materials and Methods:

All the reagents and chemicals used in the experiment was AR grade. The different planned protocol for experiments is described below.

2.1.Soil Collection and Preparation*:*

Garden soil was collected from Binaki Nagar Nagpur, Maharashtra. The soil sample was air-dried, and sieved to remove large stones and debris. The soil was divided into two portions, like normal soil and washed soil. Normal soil was used directly as the control (C1). For the preparation of nutrient depleted soil; the second portion of soil was repeatedly washed with distilled water. Washing was continued until the concentration of divalent metal ions in the soil leachate matched that of normal distilled water. The removal of divalent cations (such as Ca^{2+} and Mg^{2+}) was confirmed by using EDTA-based complexometric titration. After washing, the soil was dried at room temperature and homogenized. The prepared washed soil was used as the nutrient depleted soil as control (C2) and for nanoparticle treatment groups.

Figure 1: Preparation of wash soil for pot experiment

2.2. Removal of Divalent Cations by Complexometric Titration (EDTA) Method:

The complexometric titration method [15] was used to evaluate the removal of divalent metal cations from normal soil. The soil sample was repeatedly washed with distilled water to leach out soluble and exchangeable divalent cations such as Mg2+. After each washing cycle, the soil was allowed to settle, and the clear supernatant (filtrate) was carefully collected. The collected filtrate was then titrated against standardized 0.01 M EDTA using Eriochrome Black T as an indicator in the presence of an ammonium chloride buffer (pH 10). The washing process was repeated multiple times until the EDTA consumption for the soil filtrate matched that of the distilled water (blank). This indicated the effective removal of divalent metal cations from the soil. Eriochrome Black T (EBT) was used as indicator, which will bind with divalent metal ion and forms a wine-red coloured complex. During titration, EDTA binds strongly to the metal ions and displaces the indicator, leading to a colour change (blue). The main objective for this experiment was to make the same soil into two different conditions; one the normal soil and another one is same soil but depleted in nutrients.

2.3.Selection of washing medium for soil (Tap water vs Distilled water):

In the soil washing process the distilled water was used instead of normal (tap or groundwater) water to prevent the primarily presence of dissolved ions into the soil. The appropriate amounts of divalent metal cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and iron (Fe^{2+}), which could interfere with the objective of removing natural divalent cations from the soil. The distilled water is free from dissolved salts and metal ions, which suggesting the surety that the divalent cations originally present in the soil were leached during the washing process. For detecting the difference in between divalent cation content in distilled water and normal water for the preparation of mineral depleted soil for pot experiment, complexometric titration using EDTA was performed separately. After evaluation, the comparative analysis confirmed the

suitability of distilled water for soil washing purpose in the preparation of mineral depleted soil.

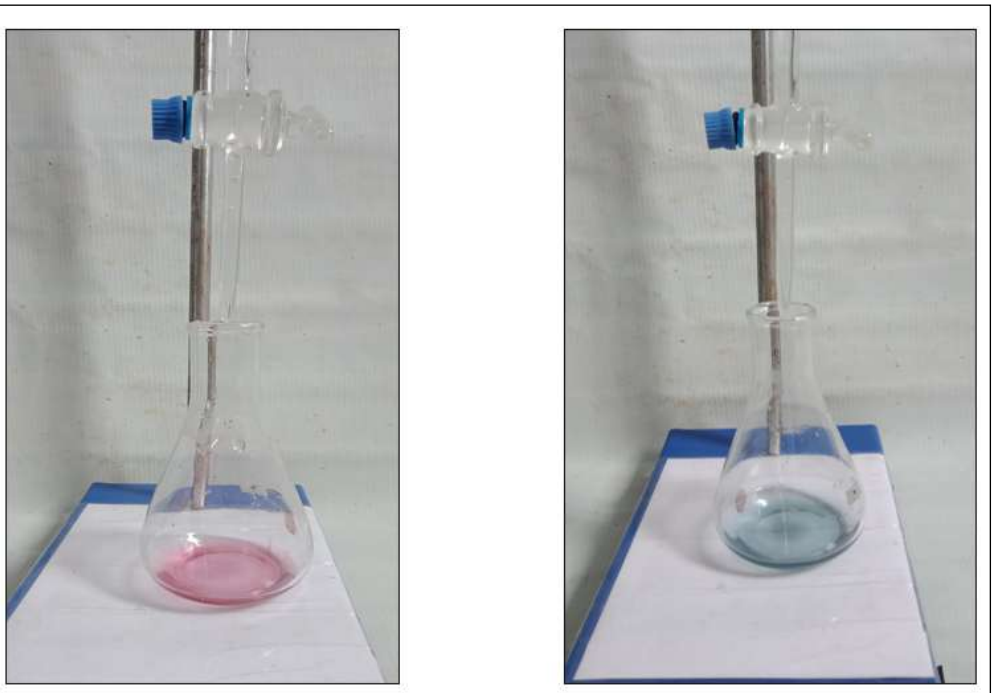

Figure 2: Complexometric titration using EDTA for divalent cation determination.

2.4.Standardization of EDTA Solution:

The solution of EDTA was standardized by using magnesium chloride solution, and the molarity was calculated using the following equation:

$$M1\ V1 = M2\ V2$$

Here,

M1 = Molarity of EDTA

M2=Molarity of Magnesium chloride

V1 = Volume of EDTA (mL)

V2= Volume of Magnesium Chloride (mL)

2.5.Determination of Divalent Cation Content in Normal Water and Distilled water:

By following the complexometric titration protocol, the distilled water and normal water are used as a sample for the selection of proper medium for soil washing process

2.6.Experimental Design and Treatment Groups

By following the complexometric titration protocol, the distilled water and normal water are used as a sample for the selection of proper medium for soil washing process.

2.7.Experimental Design and Treatment Groups

Each pot was filled with 100 g of soil. The experiment was designed with six experimental groups, including two controls (C1 and C2), synthesized and well characterized magnesium ferrite nanoparticle treatments (E1-E4) with concentration 10, 20, 40 and 80 mg/100 gram of soil. 1% NPK fertilizer was supplemented to the control group C2 and treatment group E1, E2,

E3 and E4. 30 certified spinach seeds (Spinacia oleracea L) variety of all Green – Naveen-31 was obtained from Sardar Seeds Company (ISO 9001:2015) was sowed in each pot. All the pots were maintained under identical environment condition. For the irrigation process, only distilled water was supplied to all experimental groups to avoid interference of unnecessary minerals specialty magnesium under the experimental condition. Plant growth parameters including germination rate, vigour index, plant height and plant weight [16] was evaluated after 15, 30 and 60 days of germination. The key biochemical parameters such as Protein, reducing sugar, chlorophyl and proline content was also estimated in respective days. The expected outcome from the experiment is mentioned in table 1.

<table>
<tr><th rowspan="2">Sr. No.</th><th rowspan="2">Groups</th><th colspan="4">Expected Result</th></tr>
<tr><th>Protein</th><th>Reducing sugar</th><th>Chlorophyll</th><th>Proline</th></tr>
<tr><td>1</td><td>C1</td><td colspan="3">All values can be more than other groups</td><td>The observed values can be less than the group 2</td></tr>
<tr><td>2</td><td>C2</td><td colspan="3">The observed value can be less than the group 1</td><td>The observed values can be greater than group 1</td></tr>
<tr><td>3</td><td>E1</td><td colspan="3" rowspan="4">The value can be more or less than the group 2</td><td rowspan="4">The observed values can be more or less than the Group 2</td></tr>
<tr><td>4</td><td>E2</td></tr>
<tr><td>5</td><td>E3</td></tr>
<tr><td>6</td><td>E4</td></tr>
</table>

Table 1: Table: Expected outcomes of the pot experiment

2.8. Plant Material and Growth Conditions:

For the experiment Spinacia oleracea (spinach) seeds were used. Total 30 seeds were sown uniformly in each pot of seedling tray. The tray was maintained under identical environmental conditions and watered regularly by distilled water to avoid additional mineral contamination. Plant growth parameters including germination rate, vigour index, plant height and plant weight was evaluated after 15, 30 and 60 days of germination. The key biochemical parameters such as Protein, reducing sugar, chlorophyl and proline content was also estimated in respective days.

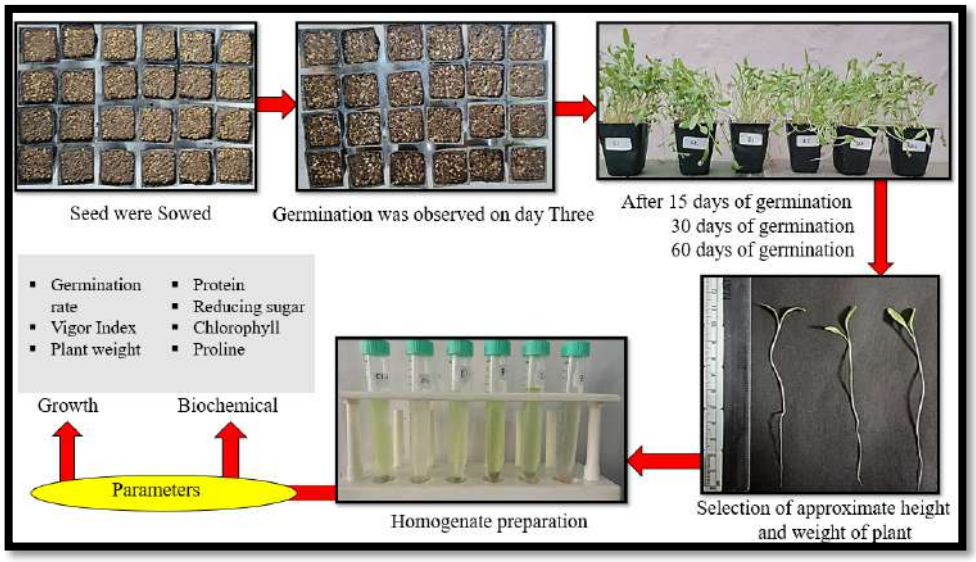

Figure 3: Stepwise representation of plant growth and biochemical analysis

2.9.Vigour Index:

For assessing the germinate success the Seedling Vigor index was calculated according to Singh A. (2020) using the fallowing formula [17].

$$Vigor\ Index = Percentage\ of\ germination \times Seedling\ length$$

2.10. Biochemical Analysis:

2.10.1. Protein Content:

Protein content was estimated by using Lowry method. After homogenization absorbance was measured spectrophotometrically using bovine serum albumin as the standard [18].

2.10.2. Reducing Sugar Content:

For the determination of reducing sugar content a standard colorimetric method (DNS method) was used. Absorbance was measured at the 510 wavelengths, and concentration was calculated using a reducing sugar standard curve [19].

2.10.3. Chlorophyl Content:

Estimation of chlorophyl was done by reported protocol followed by acetone method [20].

2.10.4. Proline Content:

Proline content was estimated using the ninhydrin method. Absorbance was measured at 520 nm, and proline concentration was calculated using a standard curve [21].

3.0. Result and Discussion:

3.1. Removal of Divalent Cations by Complexometric (EDTA) Method:

Sr. No.	Sample	Volume of EDTA (mL)	Mean	Molar Concentration of Divalent Ions
1	Normal Water	1.6	1.6	0.0032
		1.6		
		1.6		
2	Distilled Water	0.2	0.2	0.0004
		0.2		
		0.2		

3	Magnesium Chloride	5	5	0.01
		5.1		
		5		

Table 2: Divalent Cation Content in Normal Water and Distilled Water by EDTA Titration Titration

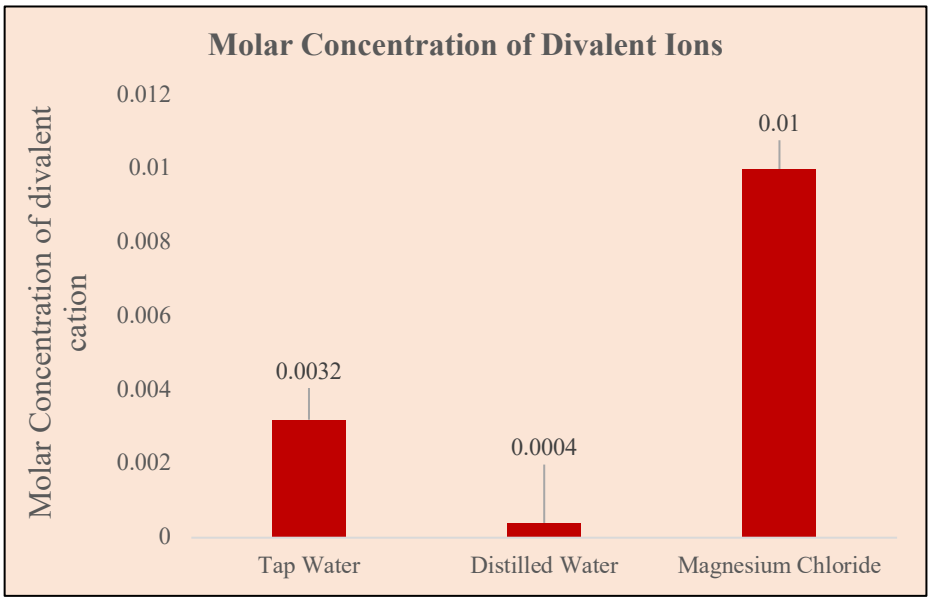

Figure 4: Divalent Cation Content in Normal Water and Distilled Water by EDTA Titration

3.2.Removal of Divalent Cations from Soil by Repeated Washing

Sr. No.	Filtrate	Mean Volume of EDTA (mL)	Molar Concentration of Divalent Ions
1	F1	2.2	0.0044
2	F2	1.9	0.0038
3	F3	1.7	0.0034
4	F4	1.4	0.0028
5	F5	1.1	0.0022
6	F6	0.9	0.0018
7	F7	0.8	0.0016
8	F8	0.6	0.0012
9	F9	0.3	0.0006
10	F10	0.3	0.0006
11	F11	0.2	0.0004
12	F12	0.2	0.0004
13	F13	0.2	0.0004

Table 3: Molar concentration of divalent cations of filtrate collected form washing cycle

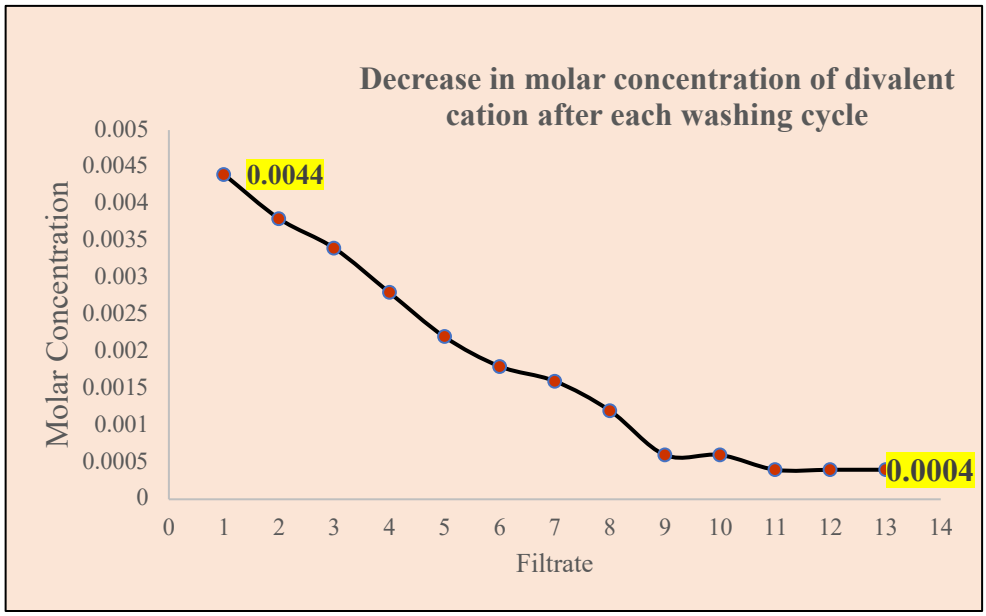

Figure 5: Molar concentration of divalent cations of filtrate collected form washing cycle

3.3.Effect of magnesium ferrite nanoparticles on seed germination rate:

Sr. No.	Experimental Group	Germination (Out of 30)	Germination %
1	C1	29.33 ± 0.58 ^{ns}	97.78
2	C2	29.00 ± 1.00 ^{ns}	96.67
3	E1	29.00 ± 1.00 ^{ns}	96.67
4	E2	28.67 ± 1.15 ^{ns}	95.56
5	E3	29.67 ± 0.58 ^{ns}	98.89
6	E4	29.00 ± 1.00 ^{ns}	96.67

Table 4: Effect of magnesium ferrite nanoparticles on seed germination

Values are expressed as mean ± SD (N = 3). Means values are followed by the superscript letter (^{ns}) are not significantly different ($p > 0.05$) according to one-way ANOVA.

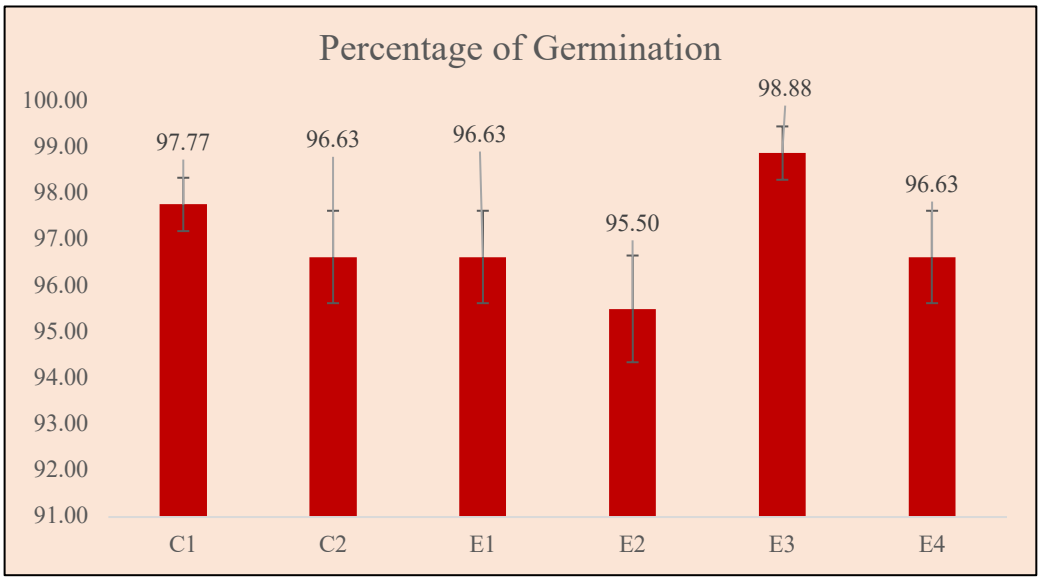

Figure 6: Effect of magnesium ferrite nanoparticles on seed germination. All values were expressed as mean ± SD (N = 3). Error bars represent the standard deviation.

It was observed that the germination rate of plants showed no significant difference between control (C1, C2) and experimental groups (E1–E4). One-way ANOVA revealed that the differences were statistically non-significant ($p = 0.88$), indicating that the treatments did not show their adverse effect on seed germination. It seems that the treatment of nanoparticles non-toxic at the germination stage and did not inhibit the germination process.

3.4.Vigor Index:

Sr. No.	Experimental Group	Vigour Index (Mean ± SD)
1	C1	1173 ± 23^{b}
2	C2	1105 ± 28^{a}
3	E1	1353 ± 46^{b}
4	E2	1512 ± 32^{b}
5	E3	1450 ± 35^{b}
6	E4	1160 ± 40^{b}

Table 5: Effect of treatment on seed vigour index

All values are expressed as mean ± SD (N = 3). Statistical comparisons were carried out by using Student's t-test where group 2 is compared with group 1 and all experimental group compared with group 2. The levels of statistical significance revealed by superscript letters indicate: (^{a}) $p < 0.05$, (^{b}) $p < 0.01$, and (^{c}) $p < 0.001$.

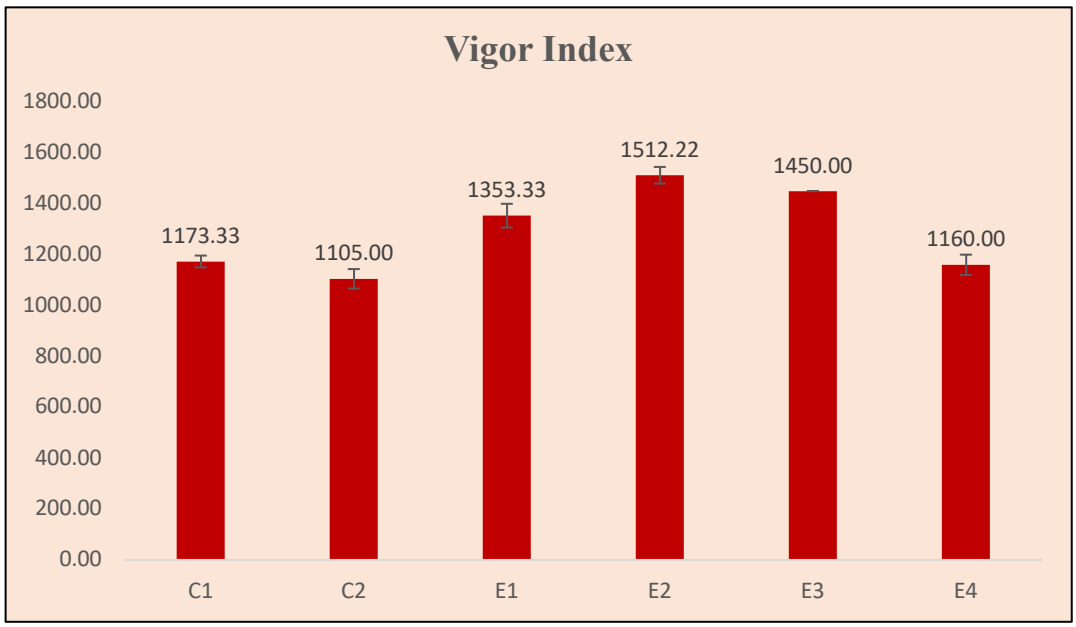

Figure 7: Effect of treatment on seed vigour index. All values were expressed as mean ± SD (n = 3). Error bars represent the standard deviation.

3.5.Plant Weight:

Sr. No.	Groups	Plant weight in gram (N=7)

		Day 15	Day 30	Day 60
1	C1	0.07± 0.014[a]	0.088 ± 0.01 [a]	0.13 ± 0.01 [a]
2	C2	0.05 ± 0.010 [a]	0.08 ± 0.01 [a]	0.10 ± 0.01 [a]
3	E1	0.08 ± 0.011 [c]	0.12 ± 0.01 [c]	0.12 ± 0.02 [ns]
4	E2	0.07± 0.01 [a]	0.12 ± 0.00 [a]	0.14 ± 0.01 [b]
5	E3	0.10 ± 0.01 [c]	0.11 ± 0.01 [c]	0.14 ± 0.01 [b]
6	E4	0.08 ± 0.014 [b]	0.14 ± 0.01 [b]	0.16 ± 0.050[b]

Table 6: Fresh plant weight at different growth stages (Day 15, Day 30, and Day 60)

All values are expressed as mean ± SD (N = 3). Statistical comparisons were carried out by using Student's t-test where group 2 is compared with group 1 and all experimental group compared with group 2. The levels of statistical significance revealed by superscript letters indicate: ([a]) $p < 0.05$, ([b]) $p < 0.01$, and ([c]) $p < 0.001$, while 'ns' denotes no statistically significant difference among the compared groups.

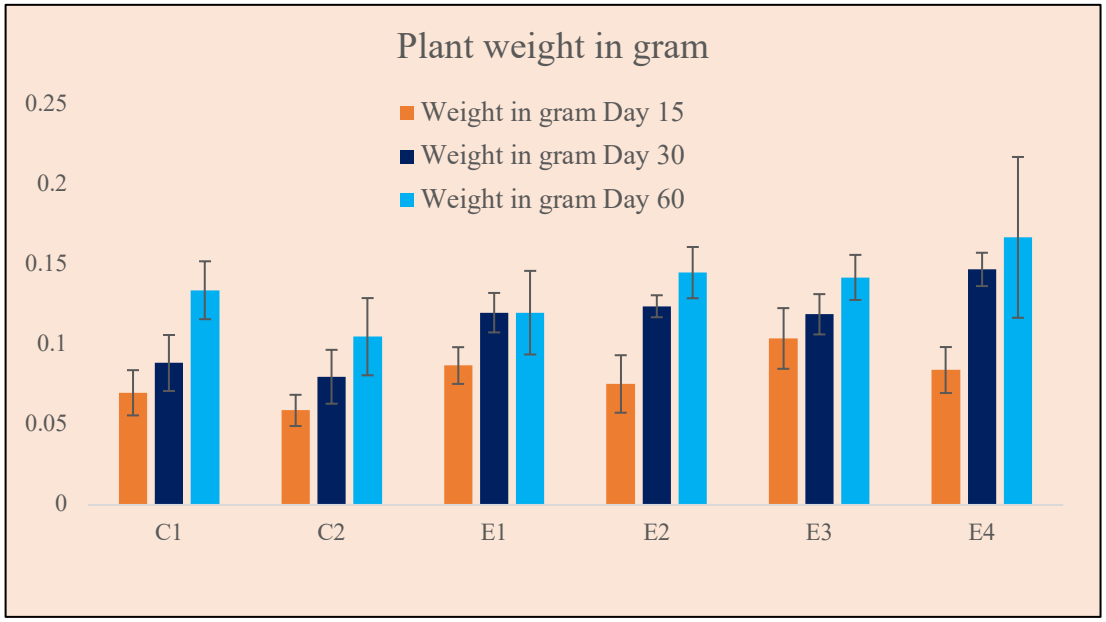

Figure 8: Fresh plant weight at different growth stages (Day 15, Day 30, and Day 60) error bars representing standard deviation (n = 7).

Plant weight was recorded at 15, 30, and 60 days after germination to evaluate growth progression. The significant decrease in weight was observed in group 2 (C2- wash soil) as compare to group 1 (C1-Normal soil) during all stages (15, 30 and 60 days) of germination.

At 15 days, the experimental groups E1 - E4 showed a significant increase in plant weight compared to control C2.

At 30 days, increased in plant weight was observed in E1, E2, E3, and E4, signifying improved growth under experimental treatments proving the dose dependent manner effect.

At 60 days, experimental groups E2, E3, and E4, revealed significantly higher plant weight than control groups (C2), suggesting a continuous positive effect of the treatment on plant biomass accumulation with time.

3.6.Biochemical analysis:

To evaluate the biochemical response of plants during growth and development, the key biochemical parameters like protein, reducing sugar, chlorophyll, and proline contents were

estimated at 15, 30, and 60 days after germination. The observed differences during the experimental process at different growth stages are presented in the following tables and graphical representations.

The significant difference of protein, reducing sugar, chlorophyll and proline concentration was observed in between two control group i.e., group 2 (C2- wash soil) and group 1 (C1- Normal soil) during all stages (15, 30 and 60 days) after germination. The concentration of protein, reducing sugar and chlorophyll content was significantly decrease in C2 as compare to C1 at different growth stages. Whereas the stress response was clearly observed during experimental period after estimating the proline concentration in C1, C2 and all treatment group. The increase in proline concentration in C2 group showed a positive stress response as compare to group C1.

3.7.Effect of magnesium ferrite nanoparticles on protein concentration in plants at different growth stages:

Sr. No.	Groups	Concentration of protein mg/gm of sample		
		Day 15	Day 30	Day 60
1	C1	12.24 ± 0.87^{b}	19.511 ± 2.18^{b}	28.07 ± 2.90^{a}
2	C2	8.072 ± 0.53^{b}	13.94 ± 0.97^{b}	20.47 ± 0.85^{a}
3	E1	15.8 ± 0.64^{c}	19.27 ± 2.28^{c}	2.27 ± 0.52^{a}
4	E2	11.51 ± 0.34^{c}	17.50 ± 2.02^{c}	23.59 ± 1.88^{c}
5	E3	10.83 ± 1.48^{b}	14.77 ± 0.74^{b}	23.33 ± 6.41^{ns}
6	E4	10.47 ± 0.24^{b}	15.25 ± 2.97^{b}	34.93 ± 9.59^{a}

Table 7: Effect of magnesium ferrite nanoparticles on protein concentration in plants at different growth stages. All values are expressed as mean ± SD (N = 3). Statistical comparisons were carried out by using Student's t-test where group 2 is compared with group 1 and all experimental group compared with group 2. The levels of statistical significance revealed by superscript letters indicate: (^{a}) $p < 0.05$, (^{b}) $p < 0.01$, and (^{c}) $p < 0.001$, while 'ns' denotes no statistically significant difference among the compared groups.

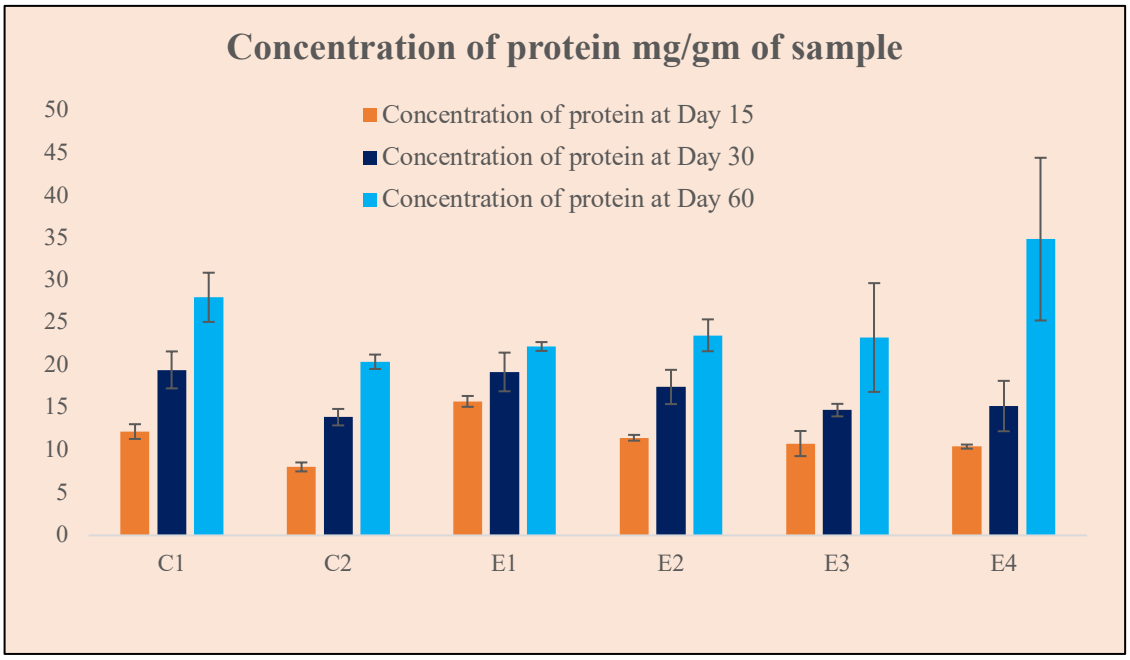

Figure 9: Effect of magnesium ferrite nanoparticles on protein concentration in plants at different growth stages

The increase in protein concentration was clearly observed in plant across all groups at different stages. At 15 days of germination, all the treatment group values show the significantly increase in concentration of protein as compare to group C2. Ampong all the treatment group E1 exhibited the highest protein content, indicating enhanced metabolic activity at the early growth stage.

By 30 days, protein concentration further increased in all treatments, with experimental groups maintaining higher values than the controls.

At 60 days after germination, a considerable increase in protein concentration was observed in all treatment groups as compare C2, particularly in E4, signifying the maximum protein concentration, suggesting a noticeable effect of the treatment on protein biosynthesis during different stages of plant development.

3.8.Effect of magnesium ferrite nanoparticles on reducing sugar content in plants at different growth stages:

Sr. No.	Groups	Concentration of reducing sugar mg/gm of sample		
		Day 15	Day 30	Day 60
1	C1	2.463 ± 0.389^{a}	1.03 ± 0.13^{a}	3.65 ± 0.13^{b}
2	C2	1.058 ± 0.246^{c}	0.46 ± 0.045^{a}	2.18 ± 0.04^{b}
3	E1	2.103 ± 0.303^{c}	0.9505 ± 0.2^{a}	2.69 ± 0.21^{a}
4	E2	2.050 ± 0.129^{c}	0.8440 ± 0.22^{a}	3.28 ± 0.22^{a}
5	E3	2.053 ± 0.466^{a}	0.6517 ± 0.24^{ns}	3.21 ± 0.24^{ns}
6	E4	1.956 ± 0.192^{c}	0.6532 ± 0.06^{a}	2.41 ± 0.06^{ns}

Table 8: Effect of magnesium ferrite nanoparticles on reducing sugar content in plants at different growth stages. All values are expressed as mean ± SD (N = 3). Statistical comparisons

were carried out by using Student's t-test where group 2 is compared with group 1 and all experimental group compared with group 2. The levels of statistical significance revealed by superscript letters indicate: ([a]) $p < 0.05$, ([b]) $p < 0.01$, and ([c]) $p < 0.001$, while 'ns' denotes no statistically significant difference among the compared groups.

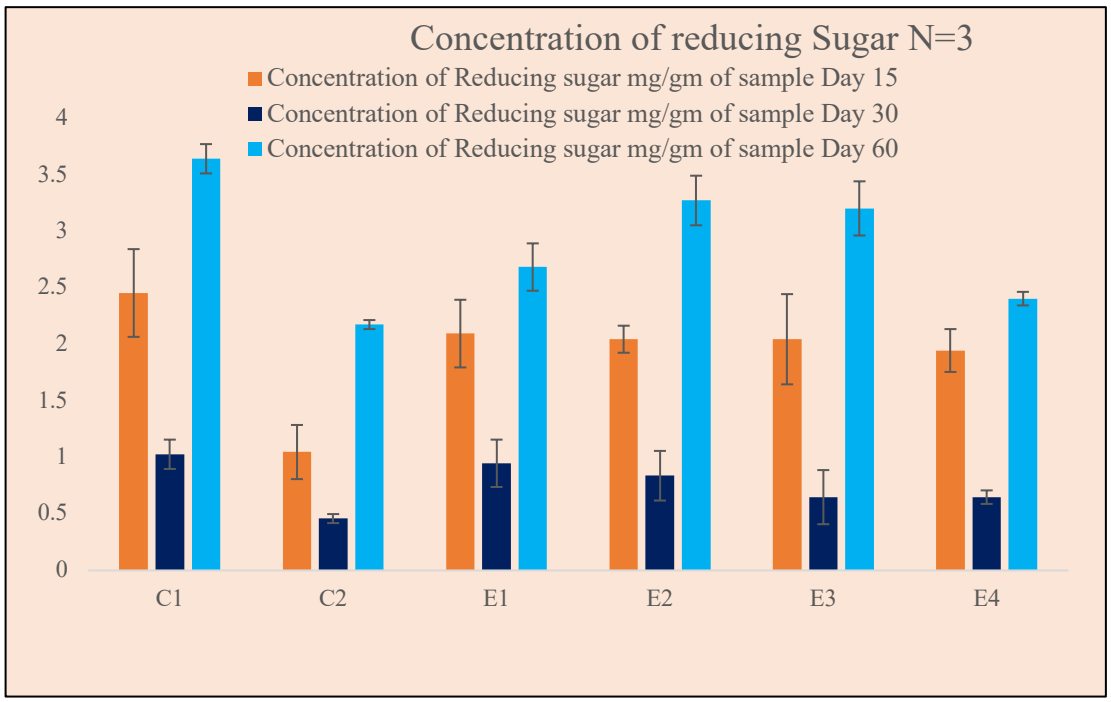

Figure 10: Effect of magnesium ferrite nanoparticles on reducing sugar content in plants at different growth stages.

The significantly lower reducing sugar content in C2 compared to C1 shows a nutrient depletion caused by soil washing, leading to reduced photosynthetic activity and carbohydrate synthesis. The treatment groups partially restored reducing sugar levels, especially at later growth stages, suggesting enhanced nutrient availability and metabolic activity.

At 15 Days, plants grown in normal soil (C1) showed the highest reducing sugar content (2.463 ± 0.389 mg/g), in spite of this a significant reduction was observed in washed soil (C2) (1.058 ± 0.246 mg/g). The experimental groups (E1–E4) revealed reducing sugar levels comparable to or slightly lower than C1 and higher as compare to C2, indicating particles show their positive effect on early carbohydrate metabolism.

At 30 Day, the overall decrease in reducing sugar concentration was observed across all groups, which conveying to increased utilization of carbohydrates during active vegetative growth. There was not significant variation was observed among most treatments, indicating uniform metabolic demand during this growth stage.

At Day 60, observable reducing sugar content increased in all groups. The highest reducing sugar concentration was recorded in C1 (3.65 ± 0.13 mg/g), while C2 showed comparatively lower values (2.18 ± 0.04 mg/g). Among the nanoparticle-treated groups, E2 and E3 demonstrated higher reducing sugar accumulation, suggesting improved photosynthetic efficiency and carbohydrate synthesis due to nanoparticle supplementation.

3.9.Effect of magnesium ferrite nanoparticles on reducing sugar content in plants at different growth stages:

	Groups	Concentration of Chlorophyll mg/gm of sample

Sr. No.		Day 15	Day 30	Day 60
1	C1	3.08 ± 0.70 [a]	4.08± 0.65 [a]	9.88 ± 0.94 [b]
2	C2	1.43 ± 0.16 [a]	2.82 ± 0.36 [a]	5.37 ± 1.20 [b]
3	E1	2.831 ± 0.42 [b]	7.78 ± 1.100 [b]	9.20 ± 1.46 [a]
4	E2	3.053 ± 0.50 [b]	4.87 ± 0.63 [c]	8.41 ± 2.61 [ns]
5	E3	3.2633 ± 0.88 [a]	5.76 ± 1.16 [b]	11.53 ± 0.88 [b]
6	E4	2.28 ± 1.03 [ns]	6.73 ± 0.00 [c]	8.97 ± 0.85 [b]

Table 9: Effect of magnesium ferrite nanoparticles on Chlorophyll content in plants at different growth stages. All values are expressed as mean ± SD (N = 3). Statistical comparisons were carried out by using Student's t-test where group 2 is compared with group 1 and all experimental group compared with group 2. The levels of statistical significance revealed by superscript letters indicate: ([a]) $p < 0.05$, ([b]) $p < 0.01$, and ([c]) $p < 0.001$, while 'ns' denotes no statistically significant difference among the compared groups.

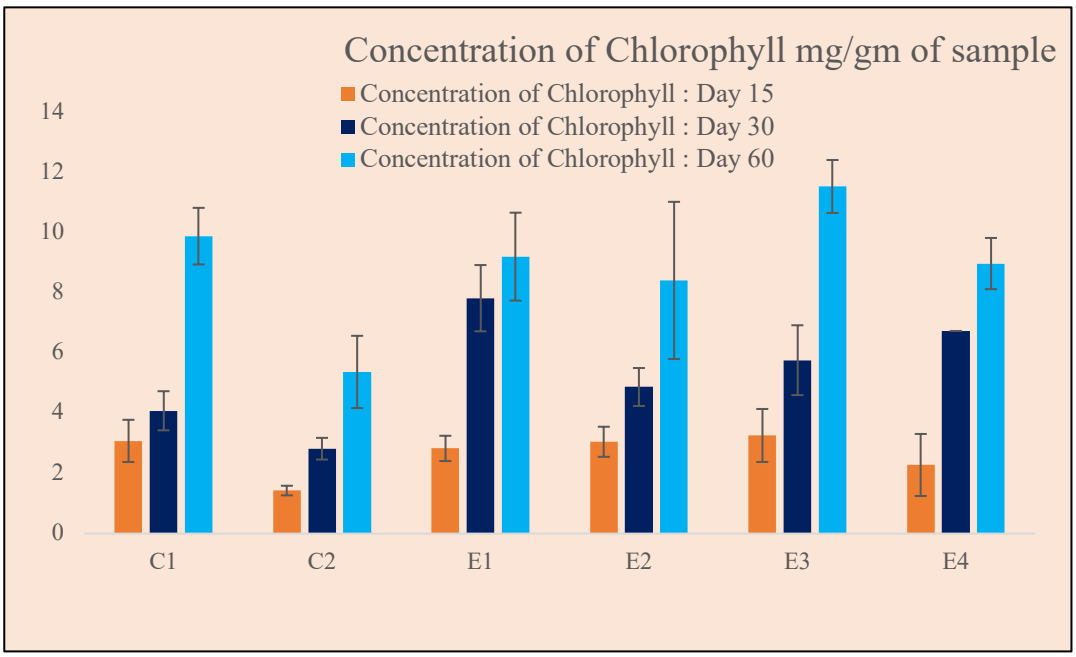

Figure 11: Effect of magnesium ferrite nanoparticles on Chlorophyll content in plants at different growth stages

At Day 15, plants grown in normal soil (C1) revealed higher concentration of chlorophyll (3.08 ± 0.70 mg/g) as compared to washed soil (C2), which showed a clear reduction of chlorophyll (1.43 ± 0.16 mg/g). The treatment group (E1–E4) showed higher chlorophyll values as compare to C2, indicating an early positive influence of nanoparticles on chlorophyll synthesis.

At Day 30, a considerable increase in chlorophyll content was observed in treatment groups. The treated group E1 (7.78 ± 1.10 mg/g) and E3 (5.76 ± 1.16 mg/g) observed significantly higher chlorophyll levels as compared to C2. Treated group E2 and E3 also shows a higher chlorophyll content as compare to group C2.

At Day 60, the accumulation of chlorophyll increased further in all groups. Plants grown in group C2 continued to revealed the lower chlorophyll content (5.37 ± 1.20 mg/g) compared to normal soil (C1). Among treated group, E3 showed the highest chlorophyll concentration

(11.53 ± 0.88 mg/g), indicating constant stimulation of chlorophyll synthesis by magnesium ferrite nanoparticles at later growth stage.

3.10. Evaluation of Plant Stress through Proline Content Estimation:

Sr. No.	Groups	Concentration of Chlorophyll ug/gm of sample		
		Day 15	Day 30	Day 60
1	C1	0.29995 ± 0.04^{c}	0.1644 ± 0.004^{c}	0.16 ± 0.01^{b}
2	C2	0.9162 ± 0.13^{c}	0.3575 ± 0.02^{c}	0.38 ± 0.01^{b}
3	E1	0.2239 ± 0.035^{c}	0.1676 ± 0.003^{c}	0.18 ± 0.05^{a}
4	E2	0.373 ± 0.020^{c}	0.1666 ± 0.003^{c}	0.14 ± 0.02^{a}
5	E3	0.2616 ± 0.104^{c}	0.1589 ± 0.023^{c}	0.11 ± 0.01^{a}
6	E4	0.3280 ± 0.121^{c}	0.1424 ± 0.0111^{c}	0.14 ± 0.01^{a}

Table 10: Evaluation of Plant Stress through Proline Content Estimation. All values are expressed as mean ± SD (N = 3). Statistical comparisons were carried out by using Student's t-test where group 2 is compared with group 1 and all experimental group compared with group 2. The levels of statistical significance revealed by superscript letters indicate: (^{a}) $p < 0.05$, (^{b}) $p < 0.01$, and (^{c}) $p < 0.001$, while 'ns' denotes no statistically significant difference among the compared groups.

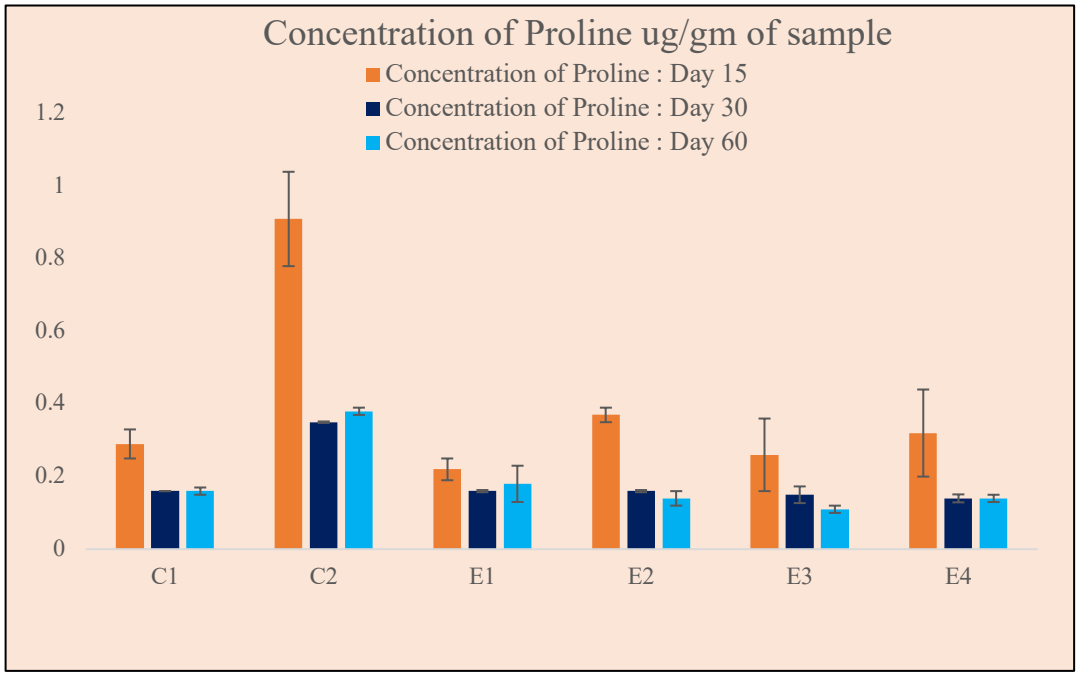

Figure 12: Evaluation of Plant Stress through Proline Content Estimation.

At Day 15, plants grown in group C2 showed higher proline concentration (0.9162 ± 0.13 ug/g) compared to group C1, signifying increase in stress due to mineral loss caused by soil washing. All experimental groups treated with magnesium ferrite nanoparticles (E1–E4) showed significantly lower proline levels than C2, suggesting reduced stress conditions.

At Day 30, a decrease in proline content was observed in all the groups. In spite of this group C2 continued to show higher proline accumulation (0.3575 ± 0.02 ug/g) compared to C1 and treated groups. The experimental treated group show low proline as compare to C2.

At Day 60, proline content remained highest in the group C2, while all nanoparticle-treated groups (E1–E4) recorded significantly lower proline levels. This reduction in proline

accumulation suggests improved physiological stability and reduced stress in plants exposed to magnesium ferrite nanoparticles over prolonged growth periods.

4. Conclusion:

The nutrient depleted soil was successfully prepared by continuous washing the normal soil by distilled water. The rapid decrease in the concentration of molar ion in each washing cycle indicates the positivity of the objective clearly. Apart from this, the decrease in the nutrient content in plant grown in C2 group shows the clear effect of alteration of soil as compare to C1 group. While the nanoparticle treatment given to the C2 group in (E1- E4) shows the increase in nutrient content highlighting the favorable effect of nanoparticle treatment on plant. The study clearly demonstrates that nutrient depletion induced by soil washing significantly affects plant growth and metabolic performance. Plants grown in washed soil exhibited reduced fresh biomass, chlorophyll, protein, and reducing sugar contents, along with increased proline accumulation, indicating nutrient deficiency–induced physiological stress. These observations confirm the essential role of soil-available nutrients in maintaining normal growth and biochemical homeostasis in plants.

Further assessment revealed that supplementation of washed soil with magnesium ferrite ($MgFe_2O_4$) nanoparticles resulted in a concentration-dependent improvement in growth and biochemical parameters. The nanoparticle-treated groups showed enhanced fresh weight, photosynthetic pigment content, and primary metabolite levels, accompanied by a reduction in stress-related proline accumulation when compared to the washed-soil control. This indicates that magnesium ferrite nanoparticles are capable of partially mitigating the adverse effects of nutrient loss in washed soils.

The beneficial effects observed may be attributed to the gradual availability of magnesium and iron ions, improved nutrient uptake, and the unique nano-scale properties of ferrites that may influence plant physiological and metabolic processes. Overall, the findings highlight the potential of magnesium ferrite nanoparticles as a promising nano-amendment for improving plant performance under nutrient-deficient soil conditions can be caused due to heavy rainfall. However, further studies focusing on long-term exposure, nanoparticle–soil–plant interactions, and environmental safety are necessary before recommending their application for sustainable agricultural practices.

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