Effect of NPK Fertilizer and Nano Iron on Growth and Glycosides Contents of *Aloe vera* L.

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Abstract

To evaluate the effect of adding three concentrations of NPK fertilizer (0, 3, and 4.5 g L^{-1}) and spraying Nano-iron at four concentrations (0, 10, 20, and 30 mg L^{-1}) on growth indicators and the content of some anthraquinone glycosides in the leaves of *Aloe vera* L., a pot experiment was conducted in the wooden shelter of the College of Agriculture at Al-Muthanna University in Iraq, during the period 2024-2025, It was conducted using a Completely Randomized Design (CRD) with three replicates.. The results showed that plants treated with NPK fertilizer at a concentration of 4.5 g L^{-1} were significantly superior in all studied traits, recording the highest values in plant height (34.91 cm), number of leaves (15.19 leaf $plant^{-1}$), leaf area (92.65 dm^2 $plant^{-1}$), fresh weight of shoot (281.5 g $plant^{-1}$), dry weight of shoot (14.48 g $plant^{-1}$), aloin content (62.24 mg g^{-1}) and emodin content (36.21 mg g^{-1}). The results also showed that the plants treated with Nano-iron at concentration (30 mg L^{-1}) were superior and gave the highest mean in plant height (31.48 cm), number of leaves (14.66 leaf $plant^{-1}$), leaf area (80.53 dm^2 $plant^{-1}$), fresh weight of shoot (245.9 g $plant^{-1}$), dry weight of shoot (12.77 g $plant^{-1}$), While the plants treated with Nano-iron concentration of 20 mg L^1 showed a significant increase in alion and emodin content of their leaves, which reached (57.88 and 32.06 mg g^{-1}), respectively.

Keywords: Aloe, Aloe vera L., NPK fertilizer, Nano- iron, Glycosides

Introduction

Aloe (*Aloe vera* L.) it is a perennial succulent belonging to Liliaceae family [5]. [15] They pointed out that its original habitat it is the arid regions of Asia, Europe, East and South Africa and America. [12] Confirmed that Aloe is one of the oldest medicinal plants used for medical purposes more than 6000 years ago to treat many diseases, according to historical manuscripts written on clay tablets in the Mesopotamian civilization. The aloe plant possesses important medicinal properties and is used commercially in the cosmetics, pharmaceutical, and food industries. It is also used in many herbal preparations. Aloe is rich in important antioxidant compounds such as vitamins A, C, and E, vitamin B (thiamine), vitamin B2 (riboflavin), vitamin B12, choline, niacin, folic acid, flavonoids, polyphenols, and carotenoids [5]. Aloe is traditionally used extensively to treat acne, ulcers, urinary tract problems, and other ailments. It is also used for skin rejuvenation and in anti-aging medicine. Aloe leaf juice is used as a stomach tonic and laxative [9]. Latex contains anthraquinone glycosides, known for their laxative effect and used as a natural remedy for constipation. It is also used in the manufacture of alcoholic beverages as a bittering agent [19], [4]. Anthraquinone glycosides are important components found in aloe leaves, including aloin, emodin, isobarbaloin, barbaloin, aloe-emodin, anthranol, aloetic acid, chrysovanic acid, cinnamic acid esters, and resistanol. These compounds are used medicinally as laxatives. Aloin and emodin are also used as analgesics, antivirals, and antibacterial [13]. Plants generally require

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suitable soil for optimal growth and higher productivity, which is influenced by soil texture and overall nutrient availability. [6] Confirmed that different soil types significantly affect the growth and biomass productivity of aloe leaves. Nitrogen, phosphorus, and potassium play a crucial role in plant growth, as they are essential nutrients that determine growth and must be available in appropriate quantities to participate in all vital processes. Nitrogen participates in numerous plant processes, such as increasing vegetative growth, expanding leaf area, and improving biomass production, thus increasing crop yield. It also contributes to the production of nitrogen-containing compounds (such as chlorophyll, nucleic acids, amino acids, ATP, and plant hormones) necessary for activating biological processes, including photosynthesis and protein synthesis [10], [7]. Phosphorus is no less important than nitrogen; it is an essential element involved in the synthesis of lipids, sugars, and nucleic acids. Furthermore, it plays a vital role in photosynthesis, respiration, nitrogen fixation, and other processes [14]. Due to their slow release and efficient absorption by plants, Nano-fertilizers have recently been used as a successful alternative to conventional fertilizers. [18] Indicated that recent studies using Nano-fertilizers have shown the effective and important impact of nanoparticles on plant growth and development. Iron (Fe) is an important and essential nutrient that plays a vital role in plant growth and development through its contribution to various stages of plant biosynthetic pathways. According to [21], it is a cofactor that contributes to stimulating the activity of more than 100 enzymes involved in cell division, protein synthesis, and DNA synthesis. Iron is also an essential element in cellular metabolism through its role in chlorophyll synthesis, chloroplast development, and cytochrome formation (which is sharply reduced in iron deficiency), as well as in photosynthesis, respiration, and enzymatic redox processes [22]. Taking into consideration the above-mentioned facts, this study was conducted with the aim of determining the extent of aloe plant response to adding of NPK fertilizer and spraying with Nano-iron, and its reflection on its growth and content of the active ingredient.

Material and Methods

Experiment location and planting season

The study was conducted during the period from 10/12/2024 to 15/12/2025 and was carried out as a pot experiment in the wooden shade belonging to the College of Agriculture / Al-Muthanna University located in the city of Samawah in southern Iraq, at the coordinates 31.32139 degrees north and 45.30407 degrees east. On 10/12/2024, leaf cuttings of aloe were planted in 5 kg plastic pots filled with a mixture of soil and peat moss at a ratio of (2-1), with one seedling in each pot. Before planting the cuttings in the pots, the mixture was sterilized with a preventive fungicide. The physical and chemical properties of the soil were analyzed prior to planting at the Soil Department laboratories, College of Agriculture, University of Al-Muthanna, as shown in Table (1).

Table (1) Physical and chemical properties of potting soil before planting

Characteristics		Measurement U.	Value
pH			7.6
E.C.		ds m^{-1}	0.95
O.M		%	0.72
Available nitrogen		mg kg^{-1} soil	25.0
Available phosphorus			19.2
Available potassium			160.6
Soil	Clay	%	36.1
	Sand		17.6
	Silt		46.3
	Texture	Silty loam	

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Experiment design, cultivation and experience factors

The study was conducted as a pot experiment using a Completely Randomized Design (CRD) with three replicates. The treatments were randomly distributed among the 36 experimental units (each containing five seedlings). The experiment included studying the effect of two factors, the first of which was adding the equal NPK fertilizer (20:20:20) at three concentrations (0, 3, and 4.5 mg L^{-1}), which was symbolized by N1, N2, N3 and N4, respectively. While the second factor was spraying Nano-iron on the vegetative groups in the form of iron oxide (Fe_2O_3) nanoparticle 50 nm (Producer of a Company: US Research Nanomaterial Inc. USA) at four concentrations (0, 10, 20 and 30 mg L^{-1}) which was symbolized by S1, S2 and N3, respectively. NPK fertilizer solutions were added at the specified levels in three batches: The first one is 120 days after the date of planting the cuttings in the pots, followed by the second and third batches, with 30 days between each batch. The iron nanoparticle solutions were also sprayed in three batches. The first batch was sprayed one week after the first batch of NPK fertilizer, followed by the second and third batches 15 days apart. The spraying process was carried out using a 16-liter hand sprayer, after adding a spreader in an amount of (0.15 ml L^{-1}) to the solutions used in the early morning. The vegetative groups of plants were sprayed with the mentioned solutions until they reached the stage of complete wetness.

Studied characteristics

After the plants reached one year of age, on December 15, 2025, the growth characteristics of all five plants in each experimental unit were measured and averaged, these characteristics included plant height, number of leaves, leaf area, and both fresh and dry weights of the foliage. Subsequently, the aloin and emodin glycosides in the leaves were estimated.

Estimation of aloin and emodin content in leaves

*Preparation of alcoholic extract:

Once the leaf samples were completely dry and their weights were stable, they were ground in an electric grinder. The leaf powders from each experimental unit were then collected, mixed thoroughly, and packaged in tightly sealed, labeled plastic containers for the extraction process. To prepare the alcoholic extract, 5 grams of powder from each sample were taken and extracted using 100 mL of methanol under reflux conditions for one hour. The extracts were then filtered using filter paper and concentrated under reduced pressure. The sample was reconstituted and its volume increased to 5 mL using methanol. To avoid the possibility of chemical degradation of the samples, they were analyzed immediately after the extraction process was completed [16].

HPLC conditions:

Sample analysis was performed using a German-made SYKAM HPLC instrument equipped with a photodiode array detector (PDA) and an automatic sampling device. Separation was performed using the isothermal concentration method at 25°C on an ODS-18 column (25 cm × 4.6 mm internal diameter, Japan). The mobile phase consisted of 2% methanol and aqueous acetic acid (70:30 v/v) and was injected at a flow rate of 1 mL/min. The injection volume was 50 µL. The quantum wavelength was set to 254 nm. The quantities of the compounds aloin and emodin were identified and measured at the Environment and Water Laboratories of the Ministry of Science and Technology in Iraq.

Statistical analysis

Data were analyzed statistically using the Genstat program, and using the analysis of variance (ANOVA) method.

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The least significant difference (LSD) test was used to compare the averages of the coefficients with a probability level of 5% [2].

RESULTS

Plant height (cm)

The results in Table (2) showed that the significant effect of NPK fertilizer concentrations on plant height. Plants treated with a concentration of 4.5 g L^{-1} significantly outperformed the other concentrations, recording the highest height of 34.91 cm, compared to the lowest height in the control group (sprayed with water only), which was 22.12 cm. Results of the same table also indicated that spraying with Nano-iron had a significant effect on the height of aloe plants. Spraying at a concentration of 30 mg L^{-1} resulted in the highest average height of 31.48 cm, a difference that was not statistically significant compared to the effect of a concentration of 20 mg L^{-1}, which resulted in a height of 30.73 cm. This was in contrast to the control sample (sprayed with water only), which recorded the lowest height (22.12 cm). Results Nano-iron also showed the significant superiority of the interaction treatment (NPK fertilizer at 4.5 g L^{-1} with Nano-iron at 30 mg L^{-1}) in this trait, by giving it the highest height of 36.00 cm, compared to the control treatment, which had a lower height (20.00 cm).

Number of leaves (leaf plant^{-1})

Table (3) shows the significant effect of NPK fertilizer concentrations on this trait compared to the control treatment, as aloe plants treated with the concentration of 4.5 g L^{-1} significantly outperformed the remaining concentrations and gave the highest number of leaves (15.19 leaf plant^{-1}), while the lowest number of leaves was in the control treatment, reaching 11.03 leaf plant^{-1}. The same table indicated the significant effect of Nano-iron spraying on the total number of leaves. Plants treated with 30 mg L^{-1} recorded the maximum average (14.66 leaf plant^{-1}), that not did differ significantly with a concentration of 20 mg L^{-1}, which yielded 14.19 leaf plant^{-1}. , in comparison with the control treatment, which produced the fewest leaves (11.66 leaf plant^{-1}). The interaction among the two experimental factors was statistically significant in this trait, the interaction treatment (NPK fertilizer at of 4.5 g L^{-1} with Nano-iron at 30 mg L^{-1}) was superior and recorded the maximum leaves number (16.00 leaf plant^{-1}) , compared to the comparison treatment, which had the minimum number (9.00 leaf plant^{-1}).

Leaf area (dm^2 plant^{-1})

Table (4) shows the significant effect of NPK fertilizer adding on leaf area, as plants treated with a concentration of 4.5 g L^{-1} significantly outperformed the rest of the plants treated with other concentrations, recording a value of 92.65 dm^2 plant^{-1}, while the lowest area was recorder in control plants (35.97 dm^2 plant^{-1}). The table also shows the significant effect of spraying with Nano-iron concentrations on increasing the leaf area of aloe, the plants treated at 30 mg L^{-1} showed the largest leaf area of 80.38 dm^2 plant^{-1}, by an insignificant difference from 20 mg L^{-1}, which yielded 79.80 dm² plant^{-1}, compared to the smallest leaf area found in the control treatment plants (46.90 dm^2 plant^{-1}).Results also confirmed the significant effect of the interaction (NPK fertilizer at 4.5 g L^{-1} with Nano-iron at 30 mg L^{1}) in this trait, which gave the highest value of 106.55 dm^2 plant^{-1}, while the control treatment gave the lowest value (23.91 dm^2 plant^{-1}).

Fresh weight of shoot (g plant^{-1})

Data of Table (5) indicate that the significant effect of NPK fertilizer concentrations on the fresh weight of shoot, compared to the lowest value in the control treatment. The concentration (4.5 g L^{-1}) was significantly superior, recording a maximum value of 281.5 g plant^{-1}, with an insignificant difference from the

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concentration (3 g L^{-1}), compared to the lowest value (100.9 g plant^{-1}) that appeared in the control plants. Similarly, the different Nano-iron concentrations had a significant effect on the fresh weight of shoot compared to the control treatment which gave the lowest weight (166.8 g plant^{-1}). The two concentrations (30 and 20 mg L^{-1}) showed the highest weights (245.9 and 238.1 g plant^{-1}), respectively, with not a significant difference between them. The interaction of (NPK fertilizer at 4.5 g L^{-1} with Nano-iron at 30 mg L^{-1}) was significantly superior in this trait, as it gave the highest weight of 317.3 g plant^{-1} compared, to lowest value (83.5 g plant^{-1}) in the control treatment.

Dry weight of shoot (g plant^{-1})

Data in Table (6) showed the significant superiority of NPK fertilizer in this trait and for all concentrations added to the aloe plant. The concentration of 4.5 g L^{-1} achieved the highest weight of 14.48 g plant^{-1}, while the control treatment recorded the lowest weight of 5.20 g plant^{-1}. The same data also indicated the significant effect of spraying with different concentrations of Nano-iron on the dry weight of shoot, plants treated with 30 mg L^{-1} and 20 mg L^{-1} significantly outperformed others, recording the highest dry weights of 12.77 and 12.23 g plant^{-1} respectively, with no significant difference between them. The control treatment recorded the lowest weight of 8.47 g plant^{-1}. Data also showed the significant effect of the interaction (NPK fertilizer at 4.5 g L^{-1} with Nano-iron at 30 mg L^{-1}) by giving it the highest weight (16.52 g plant^{-1}), compared to the lowest weight (4.28 g plant^{-1}) found in control treatment.

Aloin content of leaves (mg g^{-1})

The results in Table (7) and Figures (3-8) indicated the significant superiority of the NPK fertilizer adding at a concentration of 4.5 g L^{-1} over the rest of the concentrations, thus recording the highest aloin content of 62.24 mg g^{-1} compared to the control treatment, which recorded the lowest content of 47.83 mg g^{-1}. The table results also confirmed that spraying Nano-iron at different concentrations had a significant effect on increasing the aloin content in aloe leaves. Plants sprayed with 20 mg L^{-1} showed a significant superiority in this trait, recording a high content of 57.88 mg g^{-1}, this was superior to plants sprayed with 30 mg L^{-1}, whose aloin content was 56.28 mg g^{-1}, compared to the control plants which gave the lowest content of 53.94 mg g^{-1}. The interaction between the two experimental factors also had a significant effect in increasing the aloin content, as the interaction treatment (NPK fertilizer at 4.5 g L^{-1} with Nano-iron at 20 mg L^{-1}) recorded the highest content of 65.45 mg g^{-1}, while the lowest content was recorded in the control treatment (45.82 mg g^{-1}).

Emodin content of leaves (mg g^{-1})

Data of Table (8) and Figures (3-8) indicate that the significant effect of NPK fertilizer concentrations on emodin content, compared to the lowest value in the control treatment. The concentration (4.5 g L^{-1}) was significantly superior, recording a maximum value of 36.21 mg g^{-1}, compared to the lowest value (24.47 mg g^{-1}) that appeared in the control plants. Similarly, the different Nano-iron concentrations had a significant effect on emodin content, compared to the control treatment which gave the lowest content (28.54 mg g^{-1}). The 20 mg L^{-1} concentration was superior to the other concentrations and recorded an emodin content of 32.06 mg g^{-1}, while 30 mg L^{-1} recorded (30.92 mg g^{-1}), with a significant difference between them. The interaction (NPK fertilizer at a concentration of 4.5 g L^{-1} with Nano-iron at a concentration of 20 mg L^{-1}) had a significant effect on this trait and gave a content of 37.90 mg g^{-1} compared to the lowest content (21.45 mg g^{-1}) in the control treatment.

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Table (2) Impact of NPK fertilizer and Nano- iron on plant height (cm)

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	20.00	27.00	33.27	26.75
10	22.00	28.00	35.00	28.33
20	23.00	33.80	35.40	30.73
30	23.50	34.96	36.00	31.48
Mean	22.12	30.94	34.91	
L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer	
	1.25	1.08	2.16	

Table (3) Impact of NPK fertilizer and Nano- iron on number of leaves (leaf $plant^{-1}$)

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	9.00	12.00	14.00	11.66
10	10.14	12.97	15.00	12.70
20	12.01	14.77	15.80	14.19
30	13.00	15.00	16.00	14.66
Mean	11.03	13.68	15.19	
L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer	
	0.50	0.43	0.87	

Table (4) Impact of NPK fertilizer and Nano- iron on leaf area (dm^2 $plant^{-1}$)

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	23.91	49.69	67.11	46.90
10	33.16	71.80	88.53	64.49
20	41.08	89.90	108.41	79.80
30	45.74	88.85	106.55	80.38
Mean	35.97	75.06	92.65	
L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer	
	4.69	4.04	8.13	

Table (5) Impact of NPK fertilizer and Nano- iron on shoot fresh weight (g $plant^{-1}$)

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	83.5	186.0	231.0	166.8
10	93.0	244.6	262.0	199.9
20	108.6	287.9	315.8	238.1
30	118.3	303.9	317.3	245.9
Mean	100.9	255.6	281.5	

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L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer
	13.76	11.92	23.84

Table (6) Impact of NPK fertilizer and Nano- iron on shoot dry weight (g plant^{1})

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	4.28	9.46	11.68	8.47
10	4.88	12.85	13.34	10.35
20	5.66	14.65	16.37	12.23
30	6.00	15.79	16.52	12.77
Mean	5.20	13.19	14.48	
L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer	
	0.48	0.56	0.97	

Table (7) Impact of NPK fertilizer and Nano- iron on aloin content of leaves (mg g^{-1})

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	45.82	54.77	61.24	53.94
10	46.22	52.15	59.08	52.48
20	50.33	57.88	65.45	57.88
30	48.98	56.65	63.22	56.28
Mean	47.83	55.36	62.24	
L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer	
	0.767	0.665	1.329	

Table (8) Impact of NPK fertilizer and Nano- iron on emodin content of leaves (mg g^{-1})

Nano-iron (mg L^{-1})	NPK fertilizer (g L^{-1})			Mean
	0	3	4.5	
0	21.45	28.65	35.52	28.54
10	24.55	27.45	34.56	28.85
20	26.23	32.05	37.90	32.06
30	25.65	30.24	36.87	30.92
Mean	24.47	29.60	36.21	
L.S.D 0.05	Nano-iron	NPK fertilizer	Nano-iron× NPK fertilizer	
	0.373	0.323	0.645	

DISCUSSION

The results of the study showed that fertilizing aloe plants with NPK fertilizer had a significant effect on increasing and improving all studied vegetative and chemical characteristics.

This increase was associated with increasing the concentration of the added fertilizer, as shown in Tables (2-8). This may be attributed to the provision of nitrogen, phosphorus, and potassium, which play crucial roles in growth and metabolic processes. This leads to increased leaf growth and, consequently, an increase in leaf area. With the increased availability of these nutrients, photosynthesis also increases, resulting in greater carbon production. Carbon is the foundation for the formation of primary compounds [10], [22], this, in turn, stimulates vegetative growth characteristics, as indicated in Tables 2,3,4,5 and 6. This has a positive impact on the biosynthesis of secondary metabolites, including glycosides, as shown in Tables (7 and 8). The results of the study showed that spraying aloe plants with Nano- iron had a significant effect on increasing and improving all studied traits, both vegetative and chemical as well. This increase was associated with an increase in the concentration of iron in the spray solution, as is clear in Tables (2-6). The reason for this significant effect, in addition to what was mentioned in the introduction, may be due to the major role that iron plays in stimulating the work of enzymes involved in the nitrogen fixation process and increasing the efficiency of photosynthesis and respiration, which leads to an increase in plant biomass [1]. Or it may be due to the role of iron in forming the central structure of chlorophyll, and its availability in sufficient quantities stimulates biochemical pathways, leading to increased chlorophyll and improved efficiency of photosynthesis [3]. This is also due to the participation of iron in the oxidation process that releases energy from carbohydrates and starch, converts nitrates into ammonia, and its effective role in enhancing the metabolism of nucleic acids [20] or through improving the efficiency of the photosynthesis process by increasing the concentration of chlorophyll and carotenoids pigments and increasing the absorption of nutrients [8], [17] , which was positively reflected in improving the studied growth characteristics. Results of Table (7 and 8) indicated the significant effect of foliar feeding of Nano- iron on aloin and emodin contents of aloe leaves, noting that the leaves of plants treated with 20 mg L^{-1} recorded the highest content of both. This may be due to iron's role in chlorophyll formation and photosynthesis, and its role as a cofactor for many enzymes involved in the synthesis of secondary compounds, including glycosides.

Conclusion

Our study concluded that adding NPK fertilizer and spraying Nano-iron on aloe plants positively impacted their vegetative growth characteristics, resulting in increased plant height, number of leaves, and leaf area. This in turn had a positive effect on the fresh weight of the plant's vegetative parts and consequently on its dry weight. Furthermore, the effect of these two factors led to a significant increase in the glycosides aloin and emodin contents of the plant leaves. This noticeable effect was particularly evident when adding NPK fertilizer at a concentration of 4.5 g L^{-1} and spraying with Nano-iron at a concentration of 30 mg L^{-1}.

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CONFLICT OF INTEREST

I have no conflict of interest that I need to disclose**.**

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APPENDICES

Figure 1: Stander solution of Aloin



Figure 2: Stander solution of Emodin

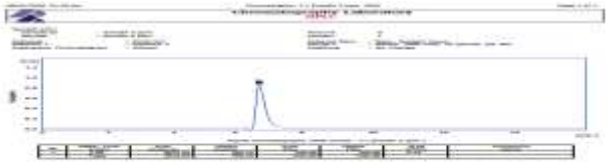


Figure 3 : Control treatment

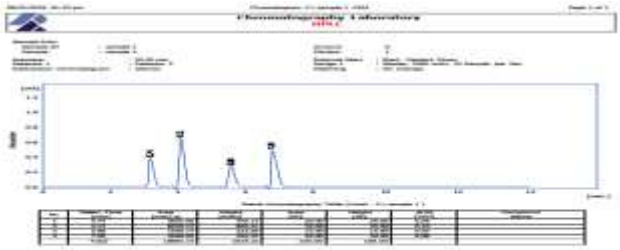


Figure 4 : NPK (0) × Iron (30 mg/L)

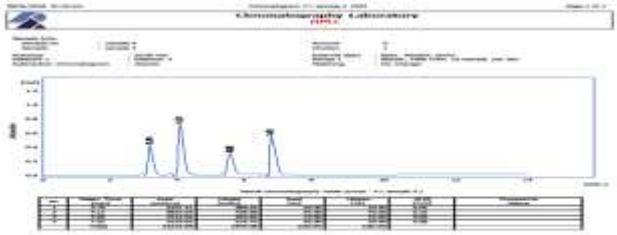


Figure 5: NPK (3g/L) × Iron (10 mg/L)

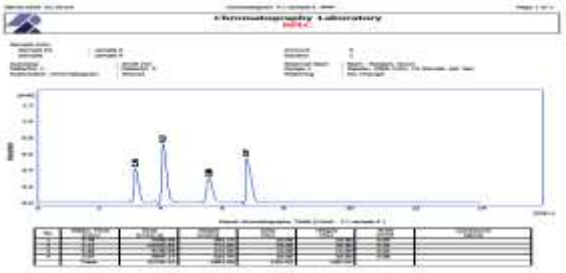


Figure 6 : NPK (3g/L) × Iron (30 mg/L)

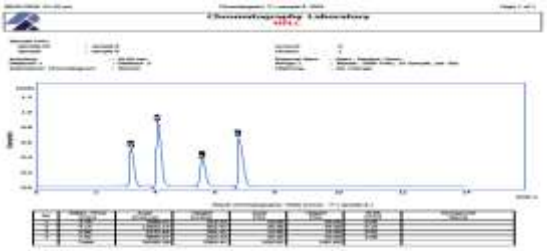


Figure 7: NPK (4. 5 g/L)× Iron (20 mg/L)

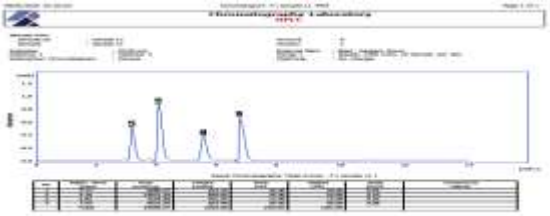


Figure 8: NPK (4. 5 g/L)× Iron (30 mg/L)

