The Impact of humic acid and Moringa leaf extract on the growth and content of certain secondary metabolites of vinca plant

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Abstract

In order to determine how adding humic acid and spraying with Moringa leaf extract will affect the growth of the vinca plant and its content of certain secondary metabolites for the fall of 2021–2022, the experiment was conducted in a nursery in Baghdad/Al-Saydiya.A factorial experiment (3×3) was conducted according to (RCBD) with three replicates,With nine treatments, , and five pots for each experimental unit, the averages were compared using the Least Significant Difference L.S.D test at the 5% probability level. The first factor involves adding humic acid to the soil at concentrations of (0, 1.5, 2.5) ml/liter, and the second factor involves spraying the plants with Moringa leaf extract at concentrations of (0, 10, 20) ml/liter.

The findings show that the humic acid treatment at a concentration of 2.5 ml/liter was considerably better in terms of the number of branches (5.13), the number of leaves per plant (36.13), the percentage of dry matter (29.37%), and the chlorophyll ratio (40.56 mg/100 gm fresh). The total amount of phenols (2.18 mg/kg), flavonoids (39.40 mg/kg), vincristine alkaloid (278.5 g/gm), and vinblastine alkaloid (347.9 g/gm) have also increased significantly. Certain vegetative growth characteristics, such as the percentage of dry matter (26.26%), chlorophyll ratio (38.31 mg/100 g fresh), total amount of phenols (2.0 mg/kg), flavonoids (38.96 mg/kg), vincristine alkaloid (234.2 g/g), and vinblastine alkaloid (273.3), are significantly improved when plants are treated by spraying with Moringa leaf extract at a concentration of 20 ml/liter.

Key words*:* *Catharanthus roseus* L, humic acid, Moringa leaves extract

Introduction

One of the Apocynaceae family's perennial decorative plants whose cultivation is successful in shaded areas is the vinca plant, *Catharanthus roseus* L. It can grow in many different types of soils and is adapted to temperate climates. It is frequently used to define the paths of interior and exterior gardens and to fill in spaces between trees (1). The vinca plant originated in Central Africa, more especially on the island of Madagascar in southeastern Africa, and in southern India and Indonesia, from where it spread to every corner of the world (2). Without concentrating on its medicinal value, it was planted in Iraq as an attractive plant in homes, parks, and public gardens.

One of the medicinal plants rich in secondary metabolites, such as phenols, flavonoids, and alkaloids, which are employed in various medical specialties is the vinca plant. There are 130 different types of alkaloids that have been found in the plant's various sections and have significant medicinal effect. It is the only plant source of these alkaloids, but roughly 11 different varieties are commercially available and utilized in the treatment of numerous diseases, particularly cancer of the ovaries, breasts, lymph nodes, and blood (3).

The availability of nutrients for the plant during the vegetative and blooming growth stages is one of the elements impacting plant growth and flowering since the plant cannot complete its life cycle without the presence of these nutrients. One of the humic substances, humic acid activates the enzymes cytochrome and oxidase, phospholipase, and phosphophorylase and acts to block other enzymes like IAA oxidase and peroxidase, fitase, and others, affecting plant growth through the processes of respiration and carbon building (4).

Through its impact on the mechanisms of crucial vital processes in the plant, such as respiration, carbon synthesis, and protein synthesis, as well as the activity of enzymes and the absorption of water and nutrients, this acid is used in very low concentrations to improve plant growth and increase yield (5). The fact that humic acid recovered from forest soil has a high percentage of IAA confirms that the impact of humic acid is very comparable to the effect of plant growth regulators (auxins, cytokinins, and gibberellins) (6).

Natural substances and hormones including cytokines, particularly Zeatin, and organic pigments like carotenoids, flavonoids, isothiocyanates, niazymycins, glucosinolates, minerals, and sterols are found in moringa leaves. All of which lead to the production of potent antioxidants. Zeatin, one of the several plant growth hormones found in abundance in morinca leaf extract. It contains micronutrients in adequate amounts and proportions that boost the development, content of active components, and production of a variety of crops (7).

Since it was discovered that moringa leaves collected from various parts of the world contain high concentrations of zeatin between 5 and 200 micrograms / g of papers, it has been proven that fresh moringa leaves contain a high percentage of zeatin. Zeatin, which belongs to natural cytocanins, works as a growth regulator for plants (8).

Aim of the study

- studying the effect of humic acid and Moringa leaves extract and their interactions on the growth of the vinca plant
- studying the effect of humic acid and Moringa leaves extract on some medicinally active constituents in the leaves of vinca plant.

Materials and methods

The experiment was carried out in a nursery in Baghdad/ Al-Saydiya for the fall season 2021-2022. The study involve two treatments, each one for three concentrations. A factorial experiment (3×3) was conducted according to (RCBD) with three replicates, , with five pots for each experimental unit. The experiment was analyzed using GenStat program, Test the least significant difference L.S.D at the level of 5%.(9).

Transactions included:

The first factor: adding humic acid to the soil

1- Comparative transaction 0: without addition and is symbolized by the symbol H0 2- adding humic at a concentration of 1.5 ml / liter and symbolized by the symbol H2

3- adding humic at a concentration of 2.5 ml / liter and symbolized by the symbol H3

The second factor: spraying Moringa leaf extract

1- Comparative transaction 0: without spraying and is symbolized by the symbol M0

2- Spraying at a concentration of 10 ml / liter and symbolized by the symbol M1

3- Spraying at a concentration of 20 ml / liter and symbolized by the symbol M2

By doing three replications; the number of transactions became nine, with five pots for each experimental unit. The seeds of the vinca plant were planted on 6/8/2021 in dishes using pitmos, and after the seedlings reached a suitable height, they were transferred to sticks with a diameter of 15 cm after filling them with pitmos at a ratio of 1:3. Adding humic to the soil in two stages, the first on 9/26/2021 and the second addition on 10/25/2021.

Moringa leaf extract was prepared by weighing 100 gm of dry leaves / 10 ml of water and grinding them with a pestle, filtering the extract with a soft cloth and secondly filtering by filter paper No. 2. The extract then diluted by water in a ratio of 32: 1 volume / volume (10) and sprayed on the plant until it was completely wet. Plant was sprayed three times, between one spray and another, 20 days, starting from the date of the first spray, 9/29/2021. Samples of homogenous plants were taken from each experimental unit at the flowering stage, specifically two weeks after the last spraying.

The measurements were taken on 4/24/2022 and the measurement parameters included:

Plant height (cm): The plant height was measured by a metric tape from the soil surface to the highest peak of the plant after the plants reached the flowering stage and the average was calculated.

Number of branches: The average number of branches for each plant in the experimental unit was calculated.

Number of leaves: The average number of leaves for each plant in the experimental unit was calculated and the average was extracted.

Percentage of dry matter % of the shoot: Five plants were extracted from each experimental unit, the root system was separated, and the shoot was air dried in a dark room for three weeks. Then, it was placed in an electric oven at a temperature of 65 degrees Celsius, and after weight stability, the percentage of dry matter was calculated as follows:

Percentage of dry matter = (dry weight) / (wet weight) x 100

Leaves content of chlorophyll: The chlorophyll content was measured using acetone 80%, as 0.2 g of the leaf was weighed and 2 ml of acetone was added and measured by a spectrophotometer at two wavelengths 663 nm and 645 nm, then the amount of chlorophyll was estimated mg.l-1 by the following equation (11):

Total Chlorophyll(mg/L)=20.2 D(645)+ 8.02 D(663)

D: wavelength

mg/100g = $mg.l^{-1}$ x final volume of extract (l) x 100/ sample weight (g)

Extraction: The extraction of phenolic compounds in the leaves was carried out according to method (12) using methanol at a concentration of 80% by the cold method, where 2 g of inflorescences and leaves were crushed in 10 ml of methanol 80% in a ceramic mortar for 5 minutes, then filtered and evaporate the solvent using a rotary evaporator at a temperature of 40 ◌̊C, the dry extract had been collected in a sealed dry bottle and kept by freezing.

Determination of phenols (mg / kg dry weight): The concentration of phenols in the leaves of the vinca plant was estimated according to the standard optical method (13) using the Folin_Denis reagent, after diluting the dried extract by adding 5 ml of distilled water, and after mixing and homogenization, 0.2 ml of The extract was added to it and 1 ml of Follen's reagent was added to it in a test tube, then 0.8 of sodium carbonate was added, its concentration is 7.5%, and after mixing the mixture well using a shaker device, it was left for 30 minutes in the laboratory atmosphere, after which the optical absorption was measured at a wavelength of 765

nm. The concentration of phenols in mg/ml was extracted from the measured extract of the phenolic compound solution of gallic acid after preparing solutions of it at a concentration of 0.1-0.1 mg/ml of distilled water and reading its optical absorption.

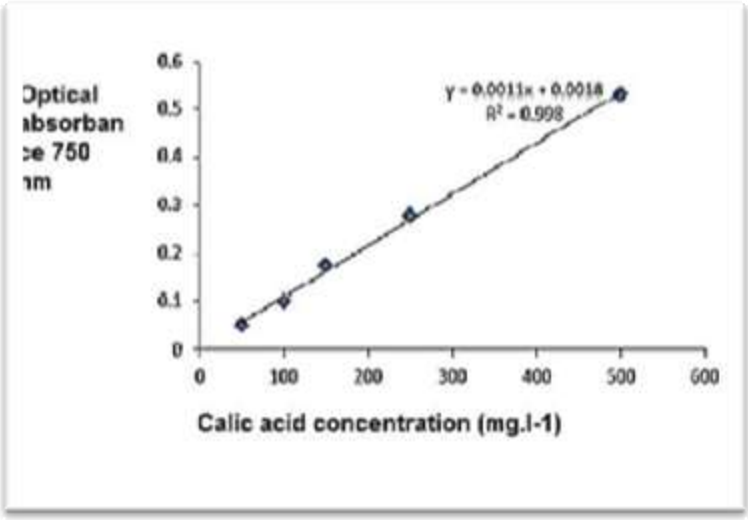

Figure 1 Standard curve for phenols

Determination of total flavonoids (mg/kg dry weight): The flavonoids were estimated according to the method used by (14) by taking 0.2 ml of the sample extract and placed in a test tube, then 2.5 ml of ethanol was added, mixed well, and 3 ml of chloride was added to it aluminum concentration of 0.01 mol / L. The test tube was left for 10 minutes in the laboratory atmosphere. The intensity of absorption was measured at a wavelength of 400 nm. Blank was prepared in the same way without adding the sample extract. The standard curve was prepared using the substance of the routine, then the straight line equation was extracted according to the figure Explained:

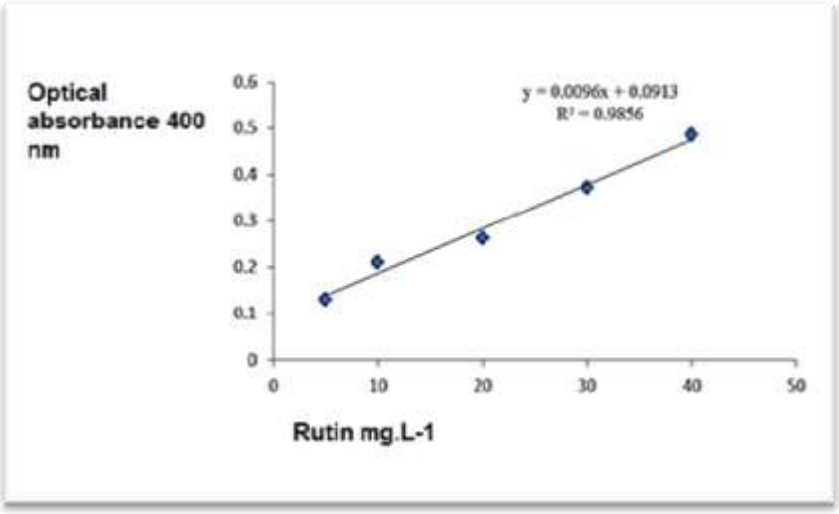

Figure 2: Standard curve for flavonoids

Extraction and quantification of alkaloids: For the aim of analyzing alkaloids, leaves samples were collected and dried at room temperature. leaves samples were crushed and preserved for analysis in plastic bags. A sensitive scale was used to weigh out 0.2 g of the sample, which was then dissolved in methanol on a water bath with an automated shaker for three hours to create the sample solution. The solution was taken out of the water bath and allowed to dry fully. The sample was dissolved in 1 ml of methanol, then it was filtered once more using filter paper. A PTFE filter needle with a 0.2 mm diameter is used, and an HPLC apparatus is used to measure the filtrate. Vincristine and Vinblastine were employed in standard solutions.

Device conditions: In the environment and water laboratories of the Ministry of Science and Technology, the alkaloids vinblastine and vincristine were assessed for the vinca plant. In order to determine the retention time and sample bundle area for both the standard solution and the sample solution, a contemporary HPLC device SYKAM-Germany was used. A column of type C18 (mm 4.6250) was used, and the mobile phase (Methanol) was pushed (NaHPO2: 77:23) at a flow rate of 1.4 ml/min. The readings were taken using normal laboratory solutions of vincristine and vinblastine at a wavelength of 220 nm and a temperature of 35 °C. The region of the unknown beams was compared to the samples' components to determine their composition.

(Form Pack Area x Standard Form Concentration)/(Standard Form Pack Area) = Sample Form Concentration x Number of Dilutions

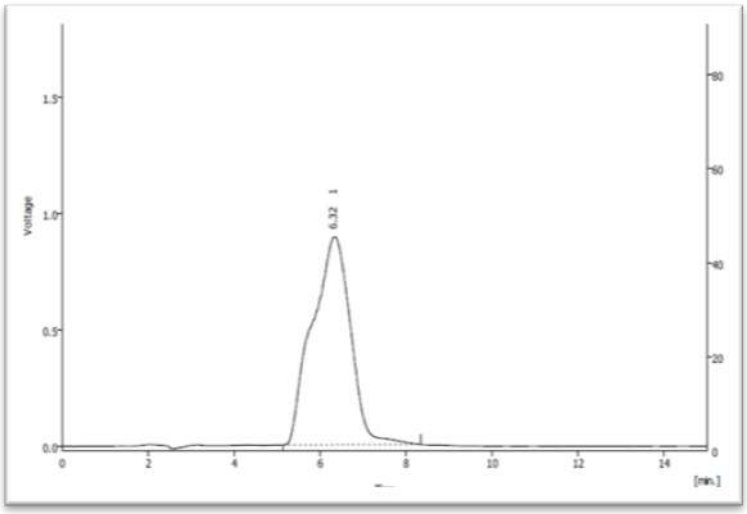

Figure 3: Standard Curved Vincristine

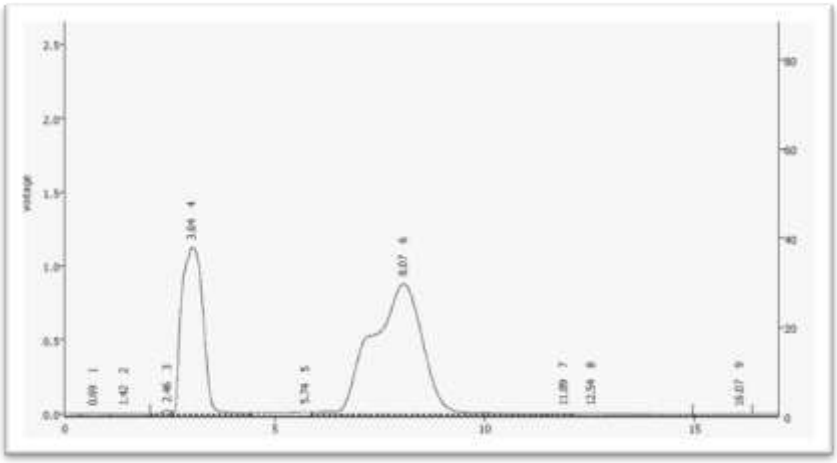

Figure 4: Standard Curve Vinblastine

Results and discussion

In comparison to the control treatment, which produced plants with an average height of 41.19 cm, the H2 treatment performed best and produced the highest height of 44.23 cm. In comparison to the control treatment, which produced the fewest branches (a total of 3.02), the H2 treatment outperformed in the number of branches of the vinca plant, amounting to 5.13. were calculated using the equation shown below:.

The humic treatments greatly increased the number of leaves per plant, although treatment H2 had the highest rate with 36.13 leaves per plant in comparison to the control treatment's lowest yield. There were 26.90 leaves on average per plant. Treatment H2 provided the highest percentage of dry matter (29.37%) among the humic acid treatments, while treatment C provided the lowest percentage of dry matter (17.91%).

The humic treatments greatly outperformed the control treatments in terms of chlorophyll content, with treatment H2 producing the greatest level—40.56—as opposed to the control treatment, which produced the lowest level—32.64. The treatments that were sprayed with the extract from moringa leaves produced plants that were noticeably taller than the control treatments. Treatment M1 produced the tallest plants, measuring 44.36 cm, while the control treatment produced the shortest plants, measuring 42.86 cm. The leaves of the plant excelled in the percentage of dry matter while spraying treatments with moringa leaf extract, and treatment M2 offered the highest percentage of 26.26% compared to the control treatment, which gave a percentage of dry matter equal to 22.33%.

In terms of dry matter percentage, humic acid treatments performed noticeably better than control treatments. The humic acid treatments clearly outperformed the control groups in terms of the vegetative growth traits they employed, as shown in Table No. 1. The materials in the samples were then identified by comparing the area of the sample's unknown beams with the recognized beam diameters of the relevant standard materials. For each sample that was diagnosed, the process was repeated under identical separation conditions (15). The chemical concentrations in the samples were calculated using the formula below: Vinblastine was it. The humic treatments greatly outperformed the control treatment in terms of the number of leaves per plant, but the H2 treatment had the highest rate, producing the highest number of branches at a rate of 3.02

branches. which gave the lowest average number of leaves of 26.90 leaves per plant.

The humic acid treatments were vastly more effective in The Humic transactions excelled morally in the chlorophyll content, as the H2 transaction gave the highest content for chlorophyll reached 40.56 compared to the treatment transaction that gave the lowest rate of chlorophyll content of 32 64. The percentage of dry matter given H2 transaction is the highest percentage of 29.37% compared to the control transaction that gave the lowest percentage of the dry matter of 17.91%. Spraying transactions at the Moringa leaf extract went beyond what was morally acceptable for the plant's height. Humic acid treatments were considerably superior in terms of the number of leaves per plant, while the greatest rate was for the control treatment, which produced the lowest rate of branches of 3.02 branches.

There were no significant differences when spraying at the Moringa leaves extract in the description of the number of branches for the plant. The number of branches in the M1 transaction was higher than the control transaction, which had the lowest height at 42.86 cm. The M1 transaction also produced the highest height at 44.36 cm. The spraying transactions outperformed the Moringa leaves extract in the description of the proportion of the dry material, whilst the leaves are for the plant. The spraying treatment with Moringa leaf extract excelled in the percentage of chlorophyll, and treatment M2 offered the greatest value for the percentage, giving a percentage of 26.26% compared to the control treatment, which gave a percentage of dry matter amounting to 22.33%.

The majority of treatments were significantly better in terms of plant height when humic acid treatment and spraying with Moringa leaf extract were combined. The H1M1 treatment produced the tallest plants, measuring 47.43 cm, while the control treatment produced the shortest plants, measuring 39.83 cm. 5.33 branches per plant was the greatest rate provided by the H2M2 treatment in terms of branch count.

In comparison to the treatment, which gave the lowest rate of the number of branches, the plant had 2.56 branches. In terms of the number of leaves, some overlapping transactions went beyond what was morally acceptable, but the H2M0 transaction gave the plant the highest rate of leaves, with 37.13 leaves, as opposed to the control transaction, which gave the lowest rate of the number.

There were 26.47 leaves for the plant, and the majority of the interference transactions excelled in their descriptions of the percentage of the dry matter and the highest percentage of the dry matter. In comparison to the control transaction, which provided the lowest percentage of dry matter at 15.57%, the H2M2 transaction achieved 30.10%, and the majority of the transactions of overlap in the percentage of chlorophyll exceeded that. However, the transaction H2M2 gave her the highest value, coming in at 41.30 compared to the control treatment, which gave her the lowest percentage of chlorophyll and came in at 30.94.

The characteristics of vegetative growth, including the height of the plant, the number of leaves, and the percentage of dry matter, which increased when leafy fertilizer with Humic acid, demonstrated that the dipal fertilizers (Humic acid) are rich in elements of nitrogen and phosphorus, which are included in the composition of proteins and enzymatic escorts that support the stimulation of the vital activities of the plant and increase cell division and building. It causes an increase in vegetative growth (16) when spraying with the extract of Moringa leaves because it is abundant in many growth hormones, especially the Zeatin, which is essential for division and elongation.(17,18)

Table (1): Effect of humic acid and Moringa leaf extract on the vegetative growth characteristics of vinca plant

Humic acid	Moringa leaf extract	Plant height	No. of branches	No. of leaves	% Dry matter	Chlorophyll percentage
H0	**M0**	39.83	2.56	26.47	15.57	30.97
	M1	42.50	2.96	26.63	17.13	32.67
	M2	41.23	3.53	27.60	21.03	34.30
H1	**M0**	43.63	4.16	27.27	23.10	36.23
	M1	47.43	4.76	28.67	24.73	37.13
	M2	46.80	4.53	30.73	27.63	39.33
H2	**M0**	45.10	4.86	37.13	28.33	40.03
	M1	43.13	5.20	35.57	29.67	40.33
	M2	44.47	5.33	35.70	30.10	41.30
L .s .d interaction		2.907	**0.631**	3.629	1.708	1.054
H0		41.19	3.02	26.90	17.91	32.64
H1		45.96	4.48	28.89	25.16	37.57
H2		44.23	5.13	36.13	29.37	40.56
L .s .d Humic acid		1.679	0.364	2.095	0.986	0.609
M0		42.86	3.86	30.29	22.33	35.74
M1		44.36	4.31	30.69	23.84	36.57
M2		44.17	4.46	31.34	26.26	38.31
L .s .d Moringa leaf extract		1.679	**N.s**	N.S	0.986	0.609

The results of Table 2 demonstrate that there is a significant presence of secondary metabolite compounds in the humic acid treatments. Treatment H2 excelled in the amount of total phenols and was equal to 2.18 mg/kg, while the control treatment was equal to 1.55 mg/kg, and the acid treatments were superior. Compared to the control treatment, which produced the lowest amount of total flavonoids at 37.00 mg/kg, treatment H2 produced the highest rate of flavonoids at 39.40 mg/kg.

The amount of vincristine alkaloid produced by humic acid treatments was exceptional; treatment H2 produced the highest

value, equal to 378.5 micrograms per gram, as opposed to the control treatment, which produced the lowest value, totaling to 167.4 µg/g. The humic acid treatments greatly outperformed the control treatment in terms of the amount of vinblastine alkaloid, with treatment H2 giving the greatest value (347.9 µg/g), compared to the control treatment, which supplied the lowest value which was 176.0 µg/g.

The M2 treatment provided the highest value for phenols, total flavonoids, vincristine alkaloid, and vinblastine alkaloid, reaching 2.0, 38.96 mg/kg, 234.2, and 273.3 g/g, respectively, compared to the control treatment, which provided the lowest. This shows that the spraying treatments using Moringa leaf extract were significantly superior in most of the secondary metabolite compounds. The values for the preceding substances were 1.82, 37.94 mg/kg, 201, and 226 g/g, respectively, while the interaction treatments between humic acid and Moringa leaf extract significantly reduced the amount of phenols and total flavonoids in most of the treatments. However, the highest treatment was provided by H2M2, which was 2.27 and 40.06 mg/kg, respectively.

The highest value was provided by the two treatments H2M2 and H2M1, which amounted to 289.6 and 289.1 µg/g, respectively, compared to the control treatment, which gave the lowest value for vincristine alkaloid amounted to 152.5 µg/g. However, some of the interaction treatments between the two experimental workers were significantly superior in the amount of vincristine alkaloid. The majority of the interaction coefficients in the amount of vinblastine alkaloid were 373.3 g/g, with the H2M2 treatment producing the highest value; in comparison. With the control treatment, which gave the lowest value for vinblastine alkaloid amounted to 162.5 µg / g.

The improvement in plant growth, the improvement in metabolic and vital efficiency, and the improvement in nutritional status when treated with humic acid and Moringa leaf extract of the plant may be responsible for the increase in secondary metabolite compounds, and consequently, the increase in the primary and secondary metabolite compounds in the production of phenols through shikimic acid (19), or because of the increase in nutrients available in the plant when foliar fertilization with Moringa leaf extract and humic acid, and thus led to an increase in flavonoids.

As a result, we draw the conclusion that the vinca plant's response to humic acid fertilization and spraying with Moringa leaf extract was reflected in plant height, the number of branches and leaves, and the percentage of dry matter, as well as in the secondary metabolite compounds represented by total phenols and flavonoids in each leaf.(20) By activating the H-ATPase enzyme in the cell membrane of cells, humic acid has the ability to increase alkaloids (21). This enzyme also increases the absorption and transfer of nutrients and amino acids through wood and bark (22) and to increase the amount of nitrogen that is primarily involved in the construction of alkaloids. This is in line with what he said.(23,24)

Table (2): Effect of humic acid and Moringa leaf extract on secondary metabolite compounds of vinca plant

Humic acid	**Moringa leaf extract**	**Phenols mg/kg**	**Flavonoids mg/kg**	**vincristine** µg/gm	**vinblastine** µg/gm
H0	**M0**	1.55	35.90	152.5	162.5
	M1	1.70	37.43	170.3	177.7
	M2	1.84	37.66	179.4	187.8
H1	**M0**	1.86	38.66	193.7	197.4
	M1	1.90	39.22	218.8	2103
	M2	2.06	39.17	233.5	265.3
H2	**M0**	2.04	39.26	256.8	318.1
	M1	2.26	38.86	289.1	352.3
	M2	2.27	40.06	289.6	373.3
L .s .d interaction		0.160	0.494	29.92	12.77
H0		1.70	37.00	167.4	176.0
H1		1.94	39.02	215.3	224.3
H2		2.18	39.40	278.5	347.9
L .s .d Humic acid		0..092	0.285	17.27	7.37
M0		1.82	37.94	201.0	226.0
M1		1.95	38.50	226.1	246.8
M2		2.0	38.96		273.3
L .s .d Moringa leaf extract		0.092	0.285	17.27	7.37

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