Effect of Organic and Chemical fertilizers on growth and active constituents of Dodonaea viscosa

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Research Article

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Abstract

Dodonaea viscosa, a common plant in northern Iraq, is recognized for its ornamental and medicinal value. This study was conducted to evaluate the effects of two types of fertilizers: organic (humic acid, HA) at four levels (0, 0.75, 1.5, and 2 $mL \cdot L^{-1}$) and chemical (NPK 20:20:20) at three levels (0, 0.5, and 1 g per pot). Growth parameters, including plant height, leaf number, branch number, stem diameter, fresh and dry weight, total chlorophyll, nitrogen (N), phosphorus (P), potassium (K) content, protein, and total phenolic acids, were assessed. Vegetative growth traits were significantly improved by foliar application of HA at 2 $mL \cdot L^{-1}$ combined with NPK at 0.5 g per pot, except for nutrient content (NPK) and protein, which increased at NPK 1 g per pot. The study also aimed to quantify total phenolic compounds and evaluate their antioxidant activity using the DPPH assay, revealing that *D. viscosa* leaves contain high levels of phenolics and act as potent natural antioxidants. The highest antioxidant activity (85.5 $\mu mol \cdot L^{-1}$) was observed with the ethyl acetate extract at 200 $\mu mol \cdot L^{-1}$. Moreover, the interaction between HA and NPK enhanced the content of specific phenolic acids, including quercetin, gallic acid, and cinnamic acid. Gas chromatography–mass spectrometry (GC-MS) analysis identified eleven compounds in the organic layer of the extract, with myrcene (43.431%), eugenol (20.122%), and α-terpineol (15.501%) being the predominant constituents. Overall, the results indicate that the optimal combination of HA and NPK not only improves growth and biomass of *D. viscosa* but also enhances the quantity and quality of secondary metabolites. However, these effects are dependent on concentration and environmental conditions, highlighting the need for further research to optimize applications in this species.

Introduction

Dodonaea viscosa L. is a flowering fast-growing shrub, which belongs to the family “Sapindaceae”. The plant can be found for the first sight in Australia and widely distributed in warmer parts of South Africa, North America and South Asia countries (Rani et al. 2009). *D. viscosa* is widely cultivate as hedges and fences because it’s desirable for this purpose and propagated by seeds and cutting (Al-Sultan et al. 1992). *D. viscosa* is a popular medicinal plant, leaves are simple, alternate, elongated or sometimes spoon-shaped, with a full edge, clustered at twig ends. The flowers are small, single-sex, greenish in color, gathered in bunches, they are useless in making decorations, leaves are used as anti-inflammatory, antibacterial and antifungal agents, also traditional leaves swellings, aches and can be used traditionally as an anti-spasmodic agent, roots and leaves as Ointment and lotion made from different plant parts to treat wounds, burns, and sprains (Teshome et al. 2010 & Zhu et al. 2017), D. *viscosa* contains many active constituents such as fixed oil, phenolics, alkaloids, flavonoids, fats, reducing sugar, steroids, saponins, tannins, gums, carbohydrates and glucosides. Therefore, flavonoids, phenols, antioxidant and terpenoids were present in the aqueous methanolic and ethyl acetate extracts of all parts of *D. viscosa*, the methods used for isolating new compounds for the plants of *Dodonaea.* species include serial extraction (Rani et al. 2009, Al-Snafi 2017). Phenols are essential secondary metabolites for most medicinal plant. These phenols can use as treatment for neurodegenerative an anti-inflammatory, anti-cancer, cardiovascular diseases, osteoporosis, and diabetes mellitus (Egamberdieva et al. 2017).

In recent decades, the rapid growth of the global population, coupled with the limitations of natural resources, has intensified the focus on developing sustainable agricultural strategies, with one of the central approaches being the efficient utilization of fertilizers to enhance both crop yield and quality.

Foliar spraying is an important method to provide the plant with the main and secondary nutrients as well as other chemicals such as growth regulators, and most plants have the ability to absorb these substances by leaves, which is more efficient and ensuring for the plant compared with the adding to the soil method, that cannot be absorbed by roots when the soil conditions are inappropriate (Murtic et al, 2012).In this context, the combined application of mineral fertilizers (NPK) and organic amendments such as humic acid (HA) has been recognized as an effective strategy to improve fertilizer use efficiency and enhance the production of both primary and secondary metabolites in plants (Seadh et al., 2012; Khalaf, 2023).

Humic acid (HA) is a major component of soil humic substances, consisting of molecules with complex structures and active carboxyl and phenolic groups. These characteristics make it an effective biostimulant that can enhance root growth, nutrient uptake, and plant tolerance to various stresses (Noroozisharaf & Kaviani, 2018). Formed through the long-term decomposition of organic residues, HA possesses a high cation exchange capacity (CEC) and the ability to form complexes with nutrients due to its carboxyl and phenolic functional groups (Canellas & Olivares, 2014). This property enables HA to stabilize ammonium nitrogen, potassium, and calcium ions in the soil, preventing their leaching or volatilization and thereby improving the efficiency of NPK fertilizer use (Ampong et al., 2022). Studies, such as those conducted on cucumber, have demonstrated that HA application can promote growth, increase dry biomass, and enhance secondary metabolite pathways (Amerian et al., 2024).

On the other hand, NPK fertilizers supply the three essential macronutrients nitrogen (N), phosphorus (P), and potassium (K) that play fundamental roles in primary and secondary metabolism of plants. Nitrogen, as a major constituent of amino acids, proteins, and chlorophyll, influences vegetative growth and photosynthesis (Marschner, 2012). Phosphorus contributes to the formation of ATP and other high-energy phosphorylated compounds, energy transfer, and enzymatic phosphorylation, while potassium is involved in enzyme activation, osmotic regulation, and protein synthesis (Marschner, 2012). Maintaining and enhancing chlorophyll content is considered a key mechanism in promoting phenolic biosynthesis, since chlorophyll improves photosynthetic efficiency and provides energy and carbon skeletons for activating phenolic biosynthetic pathways (Habibi et al., 2024). Recent studies have further highlighted the role of NPK in directly improving the quality of essential oils and phenolics in medicinal plants (Dehsheikh et al., 2020). The application of NPK fertilizers has been reported to increase chlorophyll concentration as well as phenolic and volatile compounds in medicinal plants. For instance, foliar application of Nano-NPK in *Coleus aromaticus* enhanced photosynthetic performance, chlorophyll a and b levels, carotenoids, nitrate reductase activity, and the accumulation of secondary metabolites (Akhter et al., 2025). Similarly, in *Portulaca grandiflora*, the application of 100 kg ha^{-1} of NPK resulted in the highest total phenolic content and antioxidant activity (Putra et al., 2024). These findings suggest that a balanced supply of nitrogen, phosphorus, and potassium not only provides the necessary precursors but also improves photosynthesis, thereby directly contributing to higher chlorophyll, phenolic, and volatile compound production in medicinal plants.

The combined use of HA and NPK has been widely investigated in various studies. For example, research on bell pepper demonstrated that the integration of HA with NPK increased chlorophyll content, soluble sugars, and improved soil properties such as pH and nutrient (N, P, K) uptake; moreover, the synergistic effect of their combination exceeded the benefits of either applied alone (Ichwan et al., 2022). In another study, the application of HA along with NPK in sweet potato cultivation significantly improved yield (Wang et al., 2022). Furthermore, the supplementation of NPK with HA in industrial hemp enhanced the biosynthesis of secondary metabolites such as cannabinoids and terpenoids (Bernstein et al., 2019). Collectively, these findings demonstrate that HA, by improving nutrient uptake and activating biosynthetic pathways, and NPK, by supplying essential precursors and energy, exert a pronounced synergistic effect in secondary metabolite production. Thus, their combined application optimizes nutrient absorption, yield, and quality of plants.

Considering the high potential of *Dodonaea viscosa* for producing valuable phenolic and volatile compounds, and the strong evidence supporting the synergistic effects of humic acid and NPK fertilizers, the present study aims to investigate the impact of foliar application of HA combined with NPK fertilizer on growth traits of the plant under controlled greenhouse conditions, alongside a comprehensive analysis of its phenolic composition and antioxidant activity using diverse crude extracts.

Material and methods

Procedures and design

The research has been done in Horticulture department and Landscape Design / collage of Agriculture and Forestry-University of Mosul / Iraq (Mosul 36′ 20′ N, 43º 7′ 52″, E and altitude of 225 m above the sea level). For the period from 15 September 2022 to 15 October 2023 inside the lath house, the seeds took four weeks to germinate in the greenhouse, after the appearance of the fifth leaf, the seedlings were transferred in October to 25 cm diameter plastic pots containing 1:1 peatmoss and garden soil (Table 1). The seeds taken from several sites planted with a fence of Dodonia plant inside the university of Mosul (Fig. 1 and Table 2). The experiment was arranged according to randomized complete block design (RCBD) consisting of 12 experimental units, each unit containing ten pots, the averages of the traits were compared according to Duncan's test under a probability level of 5%. (Table 3) show the temperature and humidity were recorded during the research period.

Table 1: Physical and chemical analysis of soil before planting

EC Mmho cm^{-1}	$CaCo_3$ %	N	P	K	PH	TD5 (mg kg^{-1})	Texture	Clay%	Silt%	Sand%
		(mg kg^{-1})								
0.53	19.81	15.56	63.6	1.8	7.52	420	lomia sand	12	8	80

Table 2
Administrative and service units cultivated with *D. viscosa* plant using GIS

Place number	The location	Longitude (E)	Latitude (N)
1	Grape field for horticulture department	43°8'41.94"E	36°23'12.869"N
2	College of nursing	43°8'32.791"E	36°23'7.043"N
3	College of Environment	43°8'34.353"E	36°23'5.551"N
4	College of dentistry	43°8'20.674"E	36°23'2.913"N
5	presidency of the	43°8'8.212"E	36°23'4.591"N
6	Mosul University	43°7'53.171"E	36°23'3.45"N
7	Theater of Mosul University	43°8'17.717"E	36°22'57.176"N
8	The garden of student center	43°8'21.841"E	36°22'54.138"N
9	Marketing Center	43°8'38.783"E	36°22'48.059"N
10	College of Arts	43°8'37.002"E	36°22'35.729"N
11	Department of Architectural engineering	43°8'42.96"E	36°22'36.619"N

Table 3
Average of Temperature and Humidity inside the lath house

Month	Temperature (°C)		Humidity (%)
	Great	Small	
September	34.40	21.60	34
October	32.30	15.00	43
November	22.50	8.50	52
December	17.00	2.60	63
January	14.30	1.30	68
February	15.60	2.60	63
March	19.60	8.40	60
April	25.20	11.20	58
May	34.10	18.80	57
June	40.20	23.30	26
July	41.70	22.40	27.1
August	34.20	24.23	29
September	35.40	25.30	35
October	32.10	22.10	45

Temperatures and humidity were measured using a wireless hygro-thermometer.

The seedlings were treated with the following fertilizers:

1. Humic acid (HA) at four levels: (0, 0.75, 1.5, 2) ml L^{-1}. It's sprayed until wetness occurred every month, the first addition implemented during the month of November.
2. Chemical Fertilizer (Nano NPK 20:20:20) at three levels: 0, 0.5, 1 g pot^{-1}, this fertilizer was added with water irrigation (about 200 ml NPK.pot^{-1}) once a month and the first addition was done in November.

Determination of N, P, K contents

The solution was analyzed after the chemically digestion process to determine the percentage of N, P and K in fresh leaves according to Cottenie et al. (1982). N-content was described by AOAC (1990) and P and N content according to Jackson (1977) and Issac & Kerber (1971) respectively.

Total protein

The percentage of total protein in leaves was estimated According to the source AOAC (2000) and elucidated by the equations: Total protein % = N% × 6.25, the total protein of fresh leaves was respected.

Preparation of plant extracts

The extracts of plant were equipped Plant extracts were prepared by grinded 10 g of dry leaves and mixed with 200 ml of many solvents with different polarities (Hot water, Ethanol, Ethyl acetate, Chloroform, Petroleum ether) by using dark flasks with magnetic stirrer for 72 hr and the extraction process was based the degree of boiling solvent, then the extracts after 72 hr, the extracts has been filtered through filter paper (Whatman No.1), and the vestige was re-extracted with the same volume of solvents for 24 hr and evaporated under vacuum at 60ºC to estimate antioxidant activity (Grand et al. 1988).

Preparation of the extract for oil

The leaves were collected from the field, then dried in the shade and crushed in to powder with a weight about 50 g, then the powder was mixed with distilled water (500) ml in a circul flask. The distillation process was carried out throw the Clevenger tool, two hrs after boiling, we obtained the distillation water which contained the oil, then the diethyl ether was added at a rate (2×25) ml for the purpose of separating the oil (British pharmacopeia, 1980). The oil was saved at 4°C until the analysis was begun.

Oil permission

The ingredients of the oil have been diagnosed by the device GCMS – Shimadzu QP2010, injection of 1 µL from solution. The column (0.30 mm × 30 m × 0.20 µm), oven temperature was adjusted at 70°C to 210°C, temperature of injector 280°C according to the way (Adams, 2007).

Extraction of phenolic compounds

After the extraction by petroleum ether to remote the fats present of dray leaves (25 g) by using soxhelt for 3 hr at 65º C, then this extract was evaporated at 60º C until 15 ml. After evaporation, the remains of sample were re-extracted with 250 ml of absolute ethanol by using soxhlet apparatus for 72 hr at 78°C. The resulting extract was filtered and evaporated, then the concentrated crude was carried out for acid hydrolysis (5 ml of crade was mixed with 20 ml of 1N HCl for 1 hr in bath water at 100°C (Harborne, 1973). The mixture was separated with (2×25 ml) of ethyl acetate ($C_4H_8O_2$) by using separation funnel, then the upper layer was taken and identified by HPLC-Technique (Japan 2010, Sahimadzu, Lc), the column was C_{18} (4.6 × 220) mm at a flow rate 1.5 ml min^{-1}, the mobile phase consisting of water: acetionitrate (20:80) v/v, UV absorbance at 320 nm (AL-Takay 2012).

The concentration of the separated compounds was calculated according to the equation =

$$\frac{\text{Area of separated compound} \times \text{concentration of standard compound}}{\text{Area of standard compound}} \times \frac{\text{Volum of extract (10 ml)}}{\text{Weight of dry sample (25) g}}$$

Determination of total phenolic content

Total phenolic content (TPC) was estimated according to the method reported by Gao et al. (1999) by taking 0.5 g of dry leaves, and then 20 ml of ethanol was added to it and left for 24 hours and placed in the electric vibrator for three times and for five minutes each time, and the sample was filtered and 1 ml of filtrate was taken and 0.2 ml of Folin-Ciocalteu reagent, 2 ml distilled water and 1 ml of sodium carbonate solution concentration of 15% until the color changes, then the absorption was measured by the spectrophotometer at a wavelength of 765 nm and according to the following equation:

Phenols (mg g^{-1} dry weight) =

$$\frac{\text{Reading in the device} * Total\ extract\ volume * Extract\ size\ used}{Sample\ weight * 100}$$

Antioxidant Activity

Total free radical scavenging capacity of the extract was evaluated by using DPPH, which is called (2,2-diphenyl-1-picryl-hydrazyl). Its chemical formula is (C18H12N5O6). The color is blackish-greenish powder, insoluble in water (Akar et al, 2017). We weighed 15.8 mg of DPPH and dissolved in 200 ml of methanol; different concentration of the phenolic extracts was prepared in three replicates; 50,100,150,200. µ ml^{-1}, ascorbic acid was used as a control sample. Then 1 ml of DPPH solution was added to each concentration as well as sample control then the samples were incubated at room temperature measured at a wavelength of 517 nm with a spectrophotometer device of British origin with a single cell and the following equation was applied (Ren et al. 2019).

%Reduction = (AbB – Abs) AbB × 100

AbB: Absorbance of control sample

Abs: Absorbance of sample (average absorption of the three absorption values of each dilution).

Results

plant height

The results of evaluating the effects of different treatments of humic acid (HA) at concentrations of 0, 0.75, 1.5, and 2 ml L^{-1}, and NPK fertilizer at three concentrations of 0, 0.5, and 1 g pot^{-1} on plant height showed that all treatments significantly increased plant height compared with the control. The HA1.5NPK0.5 treatment exhibited the highest growth, with a 25.79% increase relative to the control, highlighting the remarkable effect of combining 1.5 ml L^{-1} humic acid with 0.5 g pot^{-1} NPK. In contrast, the HA1.5NPK1 treatment, with a 7.58% increase, showed the lowest growth compared with the control. Other treatments also demonstrated considerable changes, with HA1.5NPK0 (20.49%), HA0.75NPK1 (17.34%), and HA0NPK1 (14.16%) ranked in the following positions. The HA0.75NPK0 and HA2NPK0 treatments recorded moderate performances, with increases of 14.22% and 10.97%, respectively. The HA0NPK0.5, HA2NPK0.5, and HA2NPK1 treatments exhibited relatively lower increases of 11.31%, 7.64%, and 8.48%, respectively, compared with the control, but still confirmed the positive effects of the treatments (Fig. 2A).

Leaf number

The results of this experiment demonstrated that different treatments of humic acid (HA) and NPK fertilizer significantly increased the number of leaves in all treatments compared with the control. The HA1.5NPK0.5 treatment recorded the highest leaf number, with a 19.79% increase over the control. In contrast, the HA0.75NPK0.5 treatment exhibited the lowest effect on leaf number, with only a 3.24% increase. Other treatments also showed considerable changes, with HA1.5NPK0 (18.49%), HA0.75NPK0 (15.80%), and HA2NPK0.5 (12.87%) ranked next. The HA0NPK1 and HA1.5NPK1 treatments both resulted in an 11.23% increase, while HA2NPK1 showed a 10.96% increase, indicating moderate performance. Furthermore, the HA0.75NPK1 and HA2NPK0 treatments, with increases of 8.90% and 8.16%, respectively, along with HA0NPK0.5 (5.38%), also confirmed the positive effect of the treatments on leaf number (Fig. 2B).

Branch number

Number of plant branches were significant compared with the control. The HA1.5NPK0.5 treatment recorded the highest number of branches, with a 69.37% increase relative to the control. In contrast, the HA0NPK0.5 treatment showed the lowest effect, with a 17.03% increase. Moreover, branch number increased by 62.52% in HA0.75NPK0, 58.77% in HA2NPK1, and 48.76% in HA2NPK0.5. The HA0.75NPK1, HA1.5NPK1, and HA0NPK1 treatments demonstrated moderate effects on branch number, with increases of 42.61%, 42.12%, and 41.48%, respectively (Fig. 2C).

Stem diameter

Based on the obtained data, the combined treatments of humic acid (HA) and NPK fertilizer also significantly increased stem length across all treatments compared with the control. The HA0.75NPK0 treatment exhibited the greatest effect, with a 70.43% increase, while the HA0.75NPK0.5 treatment showed the lowest effect, with only a 10.53% increase. Furthermore, stem length increased by 56.41% in HA0.75NPK1, 52.11% in HA2NPK1, and 42.37% in both HA1.5NPK0 and HA2NPK0, placing them in the subsequent ranks (Fig. 2D).

Chlorophyll content

According to the results of this experiment, the combined treatments of humic acid (HA) and NPK fertilizer had a significant effect on the chlorophyll content of *Dodonaea viscosa*. The highest chlorophyll content was recorded under the HA1.5NPK0.5 treatment, with a 26.24% increase compared with the control, while the HA2NPK1 treatment showed the lowest response, with a 3.38% decrease. In contrast, the HA1.5NPK0 (15.52%), HA0.75NPK0 (15.15%), and HA0.75NPK1 (13.61%) treatments exhibited remarkable increases over the control. Similarly, the HA2NPK0.5 (12.47%), HA0NPK1 (11.70%), and HA1.5NPK1 (7.60%) treatments resulted in moderate improvements (Fig. 3A).

Fresh weight

Fresh weight measurements further confirmed significant differences among treatments. The HA0.75NPK1 treatment recorded the greatest effect with a 72.59% increase, closely followed by HA1.5NPK0.5 with 71.32%. Substantial increases were also observed under HA2NPK1 (62.94%), HA1.5NPK1 (56.21%), and HA2NPK0.5 (57.23%). By contrast, the HA0NPK0.5 treatment exerted the lowest influence, with only a 17% increase compared with the control (Fig. 3B).

Dry weight

The interaction effects of HA and NPK on dry weight percentage also revealed significant differences. Consistent with fresh weight, the highest increase in dry weight was observed in HA0.75NPK1 (47.52%), followed by HA1.5NPK1 (43.98%), HA1.5NPK0.5 (34.83%), HA0.75NPK0 (34.18%), and HA2NPK1 (32.29%). The lowest effect was recorded in HA0NPK0.5, with only a 10.27% increase relative to the control (Fig. 3C).

Ratio of Fresh weight / dry weight

In addition, the fresh-to-dry weight ratio was significantly affected by HA and NPK treatments. Compared with the control (2.139), the HA2NPK0 treatment exhibited the highest ratio (2.393), followed by HA1.5NPK0.5 (2.382), HA1.5NPK0 (2.350), HA2NPK1 (2.351), and HA2NPK0.5 (2.339). Conversely, the HA1.5NPK1 treatment recorded the lowest ratio (1.866). These findings highlight the differential responses of *Dodonaea viscosa* to various HA and NPK combinations in regulating the fresh-to-dry biomass ratio, underscoring their distinct roles in biomass allocation and physiological balance (Fig. 3D).

Nitrogen, Phosphor and potassium

The evaluation of the combined effects of humic acid (HA) and NPK fertilizer on the percentage change of nitrogen (N) content in the plant revealed diverse responses among treatments compared with the control. The highest increase was observed in HA2NPK1, with a 30.84% rise, while HA1.5NPK0.5 (26.81%) and both HA0.75NPK0 and HA0.75NPK0.5 (26.06% each) also showed substantial enhancements. In contrast, the lowest effect was recorded under HA0NPK1 (Fig. 4A). For phosphorus (P), the results indicated significant differences in response to the interaction of HA and NPK treatments. The HA2NPK1 treatment exhibited the greatest increase, with a 28.43% rise relative to the control. Considerable improvements were also observed in HA0.75NPK0 (25.38%) and HA1.5NPK0.5 (24.17%). Conversely, HA0.75NPK0.5 showed the least improvement, with only a 4.93% increase (Fig. 4B). Similarly, the combined treatments significantly influenced potassium (K) content. The highest increase was recorded in HA2NPK1, with a 39.17% rise compared with the control. Notably, HA2NPK0.5 (37.98%), HA1.5NPK0.5 (31.82%), and HA1.5NPK1 (30.79%) also demonstrated remarkable enhancements throughout the experiment. On the other hand, the lowest increase in potassium was observed under HA0.75NPK0.5, with only a 3.24% rise (Fig. 4C).

Protein and total phenol compounds

The findings of this study revealed that the interaction between humic acid (HA) and NPK fertilizer had a significant effect on the percentage change in protein content. The greatest increase was observed in HA2NPK1, which exhibited a 43.27% rise compared with the control. In addition, HA1.5NPK0.5 (39.96%) and HA0.75NPK0 (38.86%) also demonstrated considerable improvements. The lowest increase, however, was recorded in HA0.75NPK0.5, with only a 14.19% rise (Fig. 5A). Similarly, the combined application of HA and NPK significantly affected the percentage change in phenolic content. The highest increase in phenols was registered in HA2NPK1 (29.86%), while the lowest was observed in HA0NPK0.5 (4.58%). Other treatments, including HA2NPK0.5 (27.03%) and HA2NPK0 (23.54%), also displayed notable improvements over the control (Fig. 5B).

Correlation Matrix Analysis

The correlation matrix analysis revealed significant relationships among the evaluated physiological, biochemical, and growth traits (Fig. 6). The results indicated that Phe exhibited strong positive correlations with LN ($r = 0.75$), BN ($r = 0.61$), FW ($r = 0.48$), and TChl ($r = 0.77$). Regarding growth parameters, FW was highly correlated with DW ($r = 0.82$), BN ($r = 0.63$), and LN ($r = 0.56$). Additionally, FW showed significant positive correlations with N ($r = 0.55$), P ($r = 0.56$), and K ($r = 0.60$). Also, TPC exhibited strong positive correlations with protein content ($r = 0.68$), K ($r = 0.76$), and P ($r = 0.44$). Protein content was also strongly and positively correlated with K ($r = 0.76$), P ($r = 0.74$), and N ($r = 0.54$). Similarly, TChl showed strong positive correlations with LN ($r = 0.76$), BN ($r = 0.61$), and protein content ($r = 0.36$).

Analysis of Trait Relationships

In this study, we investigated the relationships among measured traits under different humic acid and NPK treatments in *D. viscosa* (Fig. 7). Linear regression results indicated a significant positive linear relationship between Phe with LN ($r = 0.56$) and BN ($r = 0.36$). Additionally, a notable positive relationship was observed between phosphorus content and protein content ($r = 0.54$). Moreover, K showed strong positive correlations with TPC ($r = 0.58$), P ($r = 0.40$), and protein content ($r = 0.57$).

Antioxidant Activity of D. viscosa Extracts

To assess antioxidant activity, extracts were prepared from dried leaves of *D. viscosa* using various solvents, including hot water, ethanol, petroleum ether, ethyl acetate, and chloroform (Table 4). The use of different solvents aimed to extract compounds with varying antioxidant properties, as each solvent can selectively extract specific chemical groups such as polyphenols, flavonoids, or other secondary metabolites. Extracts were obtained from 25 g of dried leaves, and the remaining solid residue of each extract was carefully measured. The results showed that the highest solid residues were obtained with ethanol (4.952 g) and hot water (3.850 g), indicating a higher extraction yield of bioactive compounds from dried leaves using these two solvents compared to the others.

Table 4
The solid residue after extraction and evaporation from 25g dry leaves.

Extract	Residue (g)
Hot water	3.856
Ethanol	4.952
Petroleum ether	0.312
Ethyl acetate	1.225
Chloroform	2.850

In this study, the efficacy of crude extracts of *D. viscosa* was evaluated using petroleum ether, chloroform, ethyl acetate, ethanol, and hot water as solvents, in comparison with a standard, at concentrations of 50, 100, 150, and 200 $\mu g\cdot mL^{-1}$ (Table 5). The results, expressed as mean values, showed a concentration-dependent increase in activity for all solvents and the standard. At 50 $\mu g\cdot mL^{-1}$, the standard exhibited the highest activity (82.3%), followed by ethyl acetate (76.8%), ethanol (73.2%), hot water (70.1%), chloroform (65.2%), and petroleum ether (71.2%). At 100 $\mu g\cdot mL^{-1}$, the activity of the standard was 83.5%, with ethyl acetate (80.7%), ethanol (75.5%), hot water (73.3%), chloroform (70.7%), and petroleum ether (74.3%) ranked subsequently. At 150 $\mu g\cdot mL^{-1}$, the standard reached 85.7%, followed by ethyl acetate (83.3%), ethanol (78.3%), hot water (75.2%), chloroform (73.9%), and petroleum ether (77.5%). At the highest concentration (200 $\mu g\cdot mL^{-1}$), the standard exhibited 87.6% activity, with ethyl acetate (85.5%), ethanol (81.5%), hot water (77.5%), chloroform (78.7%), and petroleum ether (81.7%) in descending order. Ethyl acetate and ethanol extracts performed better than the other solvents but were less effective than the standard, indicating the superior potency of the standard across all tested concentrations.

Table 5
The concentration and percentage of standard sample and crude extracts of *D. Viscosa* leaves as antioxidants (μ ml^{-1})

The concentration μ ml^{-1}	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Hot water	Standard sample
Crude Extracts						
50	71.2 d	65.2 d	76.8 d	73.2 d	70.1 d	82.3 d
100	74.3 c	70.7 c	80.7 c	75.5 c	73.3 c	83.5 c
150	77.5 b	73.9 b	83.3 b	78.3 b	75.2 b	85.7 b
200	81.7 a	78.7 a	85.5 a	81.5 a	77.5 a	87.6 a

Table 6 and Fig. 9 demonstrated that the individual application of NPK fertilizers and humic acid (HA) enhanced the concentration of phenolic compounds. Specifically, at the NPK level of 1 g per pot, quercetin reached 0.093, gallic acid 0.227, salicylic acid 0.213, and para-hydroxybenzoic acid 2.220 $mg\cdot g^{-1}$. Application of humic acid at all levels gradually increased phenolic compound concentrations, with the highest bioactive compound levels observed at 2 $mL\cdot L^{-1}$ HA, including quercetin (0.042 $mg\cdot g^{-1}$), gallic acid (0.322 $mg\cdot g^{-1}$), salicylic acid (0.062 $mg\cdot g^{-1}$), para-hydroxybenzoic acid (0.153

$mg \cdot g^{-1}$), and cinnamic acid (1.231 $mg \cdot g^{-1}$). When the interaction between NPK at 0.5 g per pot and HA at 2 $mL \cdot L^{-1}$ was examined, a significant increase in the concentrations of quercetin (0.091 $mg \cdot g^{-1}$), salicylic acid (1.022 $mg \cdot g^{-1}$), para-hydroxybenzoic acid (1.311 $mg \cdot g^{-1}$), and cinnamic acid (3.711 $mg \cdot g^{-1}$) was observed.

Table 6: Effect of NPK and humic acid on the concentration of many phenolic compounds (mg g^{-1} dry weight) of *D. viscosa*.

Treatment	Quercetin (mg g^{-1})	Gallic acid (mg g^{-1})	Salicylic acid (mg g^{-1})	p-Hydroxy benzoic acid (mg g^{-1})	Cinamic acid (mg/g)
				HA × NPK	
0×0	0.001	0.010	0.014	0.011	0.021
0.75×0	0.003	0.008	0.01	0.013	0.115
1.5×0	0.031	0.023	0.045	0.025	0.126
2×0	0.041	0.322	0.062	0.153	1.231
0×0.5	0.052	0.143	0.112	1.231	1.303
0.75×0.5	0.019	0.003	0.132	0.105	1.421
1.5×0.5	0.037	0.389	0.203	0.321	2.621
2×0.5	0.091	0.005	1.022	1.311	3.711
0×1	0.093	0.227	0.113	2.220	1.251
0.75×1	0.0014	0.017	0.145	0.103	**
1.5×1	0.003	0.073	0.080	**	0.192
2×1	0.001	0.004	0.066	**	0.204

** the absence of compound

Essential oil of D. viscosa

The essential oil extracted from *D. viscosa* was analyzed using gas chromatography–mass spectrometry (GC-MS), revealing multiple volatile compounds with varying relative percentages (Fig. 10 and Table 7). Myrcene was identified as the major and most abundant component, accounting for 43.431% of the oil. Eugenol (20.122%) and α-terpineol (15.501%) were the next most abundant compounds, and together, these three accounted for approximately 79% of the oil composition. Other notable constituents included 1,8-cineole (5.975%), benzaldehyde (4.077%), linalool (3.330%), and α-calacorene (3.015%). Minor components identified included nerol (1.221%), benzoic acid (1.214%), n-octanol (1.102%), and geraniol (1.012%). These minor compounds, such as 1,8-cineole, benzaldehyde, linalool, and α-calacorene, contribute to the chemical complexity of the oil, while trace amounts of nerol, benzoic acid, n-octanol, and geraniol were also detected.

Table 7
Permission of oil by GC-MS technology from *D. viscosa*

Peak	Name of compound	Area%	Retention time (min)
1	n-octanol	1.102	3.699
2	benzoic acid	1.214	4.678
3	linalool	3.330	5.878
4	α-terpineol	15.501	6.025
5	nerol	1.221	7.049
6	geraniol	1.012	8.296
7	α-calacorene	3.015	9.527
8	benzyldehyde	4.077	10.717
9	eugenol	20.122	13.295
10	myrcene	43.431	15.111
11	1.8-cinol	5.975	15.715
	Total	100.00	

Discussion

The combined application of humic acid (HA) and NPK fertilizers significantly influenced the growth indices of *D. Viscosa*, a resilient shrub well-adapted to diverse environmental conditions. Our results clearly demonstrated that the combined use of HA and NPK enhanced the plant height of *D. Viscosa* The observed increase in plant height aligns with previous findings indicating that humic substances promote plant growth by improving nutrient availability and soil structure (Ichwan et al., 2022). HA is recognized as a chelating agent for essential nutrients, likely contributing to the enhanced performance of specific HA-NPK combinations in this study by facilitating nutrient availability to the plant (Al-Issawi et al., 2021). Notably, the combination of moderate HA concentrations with NPK resulted in the greatest increase in plant height, suggesting an optimal balance for maximizing nutrient uptake efficiency. This finding is consistent with prior research on *D. Viscosa*, which reported that HA enhances root development and nutrient absorption, particularly when combined with mineral fertilizers (Lak et al., 2024). However, higher HA concentrations were associated with reduced growth, possibly due to altered nutrient solubility and uptake dynamics. Elevated concentrations may occasionally limit growth by causing excessive nutrient availability or altering soil pH.

The increase in leaf number across various HA and NPK treatments underscores the role of HA in enhancing the photosynthetic capacity of *D. Viscosa*. Leaves are critical for photosynthesis, and their increased number suggests that HA facilitates the uptake of essential nutrients, such as nitrogen, which is vital for chlorophyll synthesis and leaf development (Hakeem et al., 2023). Our findings indicate that the treatments likely optimized nitrogen availability, thereby supporting leaf proliferation. A significant positive correlation was observed between leaf number and the internal concentrations of nitrogen (N), phosphorus (P), and potassium (K) in the plant (Fig. 6). These results are consistent with previous studies that reported a positive effect of HA on nitrogen uptake under controlled conditions (Ichwan et al., 2022; Khalaf, 2023). Variations in leaf number responses among treatments may be attributed to the differential ability of HA to form complexes with nutrients, which varies depending on concentration and application method. For instance, foliar application of HA can enhance leaf growth by directly supplying nutrients to leaf tissues, bypassing soil-related limitations (Seadh et al., 2012; Khalaf, 2023). The positive correlation between leaf number and other growth traits in this study suggests that increased leaf area may enhance photosynthetic activity and biomass accumulation.

The increase in branch number, particularly with the optimal combination of humic acid (HA) and NPK fertilizers, highlights the role of HA in stimulating lateral growth, likely through the activation of hormonal pathways and enhanced nutrient availability. Correlation analyses revealed strong positive relationships between the increase in branch number and the internal concentrations of nitrogen (N), phosphorus (P), and potassium (K). Branching is influenced by elements such as phosphorus and potassium, which are critical for cell division and elongation (Abou El Salehein et al., 2021; Wulandari et al., 2019). The superior performance of certain treatments suggests that HA enhances the availability of these nutrients by chelating them and improving soil microbial activity (Abdel-Mawgoud et al., 2007; Ichwan et al., 2022). The strong correlation between branch number and other growth traits, such as plant height and leaf number, indicates a synergistic effect that contributes to the overall structural improvement and environmental adaptability of *D. Viscosa*.

The increase in stem diameter, concurrent with height, further confirms the efficacy of the HA and NPK combination in promoting growth. The studied treatments likely enhanced gibberellin activity and nutrient uptake, as HA is known to stimulate hormonal pathways that regulate longitudinal stem growth (Mohammed Ali et al., 2023). These authors noted that gibberellins play a significant role in increasing stem diameter and length by promoting cell division and enhancing cell elongation in the internodal regions, resulting in thicker stems. Variations in stem diameter responses among treatments may stem from differences in HA concentration and its interaction with soil properties, such as pH and cation exchange capacity, which affect nutrient availability. Research on crops like maize has shown that combining HA with phosphate fertilizers can enhance longitudinal stem growth by improving phosphorus mobility in the soil (Wang et al., 2022). The positive correlation between stem length and other growth indices in this study suggests that HA and NPK synergistically enhance the overall vigor of *D. Viscosa*. The enhancement of total chlorophyll content in *D. Viscosa* under various HA and NPK treatments underscores the role of HA in improving photosynthetic efficiency. Chlorophyll levels are strongly influenced by nitrogen availability, and the most effective treatment likely maximized nitrogen uptake, a finding consistent with studies demonstrating enhanced nitrogen absorption under HA application (Seadh et al., 2012; Khalaf, 2023; Nabi et al., 2025). However, reduced chlorophyll content in certain treatments may be attributed to high HA concentrations, potentially causing nutrient imbalances or stress, as elevated HA levels can sometimes reduce nutrient solubility (Meganid et al., 2015). This study also revealed that HA application under salt stress conditions increased growth and chlorophyll content in bean plants, but at high concentrations or under severe stress, negative effects such as reduced nutrient uptake and disrupted nutritional balance may occur. The positive correlation between chlorophyll content and other growth traits in this study highlights the critical role of photosynthetic capacity in supporting overall plant growth.

The increase in fresh and dry weight under the studied treatments reflects the cumulative effect of HA and NPK on biomass accumulation and quality. The HA and NPK treatments likely improved water and nutrient retention in the soil, leading to enhanced turgor pressure and plant growth. The ability of HA to enhance soil structure and water-holding capacity, as reported in studies on Egyptian cotton (Seadh et al., 2012), maize (Wulandari et al., 2019), and *Vitis vinifera* (Iman et al., 2020), likely contributed to these results. A strong correlation between fresh weight and other growth traits indicates that HA and

NPK collectively enhance overall plant productivity (Fig. 6). Variations in the fresh-to-dry weight ratio reflect the influence of HA and NPK on water content and biomass allocation. Higher ratios in some treatments suggest improved water retention due to HA's effects on soil structure and root development, while lower ratios indicate a shift toward greater dry matter accumulation, which is advantageous in arid environments (Yadegari, 2022).

Our results demonstrated that the increased percentages of nitrogen (N), phosphorus (P), and potassium (K) in *D. Viscosa* reflect the role of HA in enhancing nutrient uptake in the studied treatments. The simultaneous application of HA and NPK fertilizers leads to the formation of slow-release complexes with ammonium nitrogen. HA, with its carboxylic and phenolic functional groups, enhances the cation exchange capacity (CEC) of the soil and reduces nitrogen volatilization (Xu et al., 2021). These authors further noted that HA increases phosphorus (P) availability to plant roots by reducing phosphate adsorption by soil minerals and inhibiting Ca–P precipitation. Additionally, HA facilitates the storage and gradual release of K^+ in the rhizosphere by forming potassium-humate complexes, thereby enhancing K uptake by the plant (Dinçsoy and Sönmez, 2019; Xu et al., 2021). Systematic studies and meta-analyses have shown that HA improves nitrogen use efficiency (NUE) and N uptake, even when NPK fertilizers are applied (Ma et al., 2024). A strong correlation was observed between fresh and dry weight and the levels of N, P, and K. The combined application of HA and NPK increased dry weight of shoots and leaves, chlorophyll content, N and P uptake, and root enzyme activities (such as H^+-ATPase and nitrate reductase), as reported in wheat (Ampong et al., 2022) and maize (Jing et al., 2022). Furthermore, a strong correlation was noted between fresh and dry weight and the levels of N, P, and K (Fig. 6). Increased phosphorus availability typically enhances biomass production and dry weight by improving cell division and protein synthesis. As an essential nutrient, phosphorus plays a critical role in photosynthesis, energy transfer, and carbohydrate metabolism in plants, and its deficiency can limit plant growth and dry weight.

The increase in protein content is directly linked to enhanced nitrogen uptake, as proteins are nitrogen-rich compounds. Our results indicated that the application of HA and NPK significantly increased protein content. It has been reported that HA accelerates nitrogen uptake and assimilation by stimulating the activity of nitrogen metabolism enzymes, such as nitrate reductase, glutamate synthetase, and glutamate dehydrogenase, potentially leading to enhanced protein synthesis (Canellas et al., 2015). The effect of HA on protein content depends on multiple factors, including HA concentration, application method (soil or foliar), and plant species, resulting in variable outcomes under different conditions (Ampong et al., 2022). A significant correlation was also observed between protein content and the levels of N, P, and K, consistent with findings by Lamlom et al. (2023) in wheat.

The observed increase in phenolic compounds in this study indicates that HA and NPK fertilizers can enhance the production of secondary metabolites, which are critical for plant stress resistance. Humic acid promotes nutrient uptake from NPK fertilizers by activating root signaling pathways and modulating hormones such as auxin and gibberellin (Rathor et al., 2023). HA directly induces the expression and activity of key enzymes in the phenylpropanoid pathway, such as Phenylalanine Ammonia-Lyase (PAL) (Vahdati et al., 2023; Cao et al., 2025). Enhanced PAL activity accelerates the conversion of phenylalanine to phenolic acid precursors, thereby increasing the accumulation of phenols and flavonoids (Bernstein et al., 2019; Habibi et al., 2025). Conversely, balanced availability of nitrogen, phosphorus, and potassium (NPK) plays a dual role. Excessive nitrogen can alter the C/N ratio, potentially affecting the synthesis of certain phenolic compounds (Narvekar and Tharayil, 2021).

However, optimal availability of N, combined with P and K, provides the necessary resources and energy (ATP, phosphorylation, enzymatic cofactors) for secondary biosynthetic pathways, thereby enhancing the production capacity of phenolic and volatile compounds (Amarowicz et al., 2020).

Previous studies have reported that HA enhances the quality and quantity of volatile compounds (e.g., terpenoids, esters, alcohols) by complexing ions and increasing the availability of micronutrients (e.g., Mn, Fe, Zn), which serve as cofactors for antioxidant enzymes and terpenoid synthases (Noroozisharaf and Kaviani, 2018; Xu et al., 2025). Additionally, the synergy between HA and NPK is believed to induce stress-like or eustress responses (priming), leading to increased expression of genes associated with secondary metabolite synthesis and enzymes in the terpenoid and phenylpropanoid pathways (Rasouli et al., 2022). Experimental results in medicinal and aromatic plants (e.g., *Thymus*, *Cannabis*, *Coriandrum*) have shown that combining HA with NPK fertilization or partially substituting NPK with HA not only increases the quantity (total phenolic content/essential oils) but also alters the qualitative composition (relative proportions of volatile components) (Noroozisharaf and Kaviani, 2018; Rasouli et al., 2022). Therefore, a similar increase in phenolic and volatile compounds is expected in *D. Viscosa*, although species-specific experiments are needed to confirm these effects.

The results of this study clearly demonstrate that the application of crude leaf extracts of *D. Viscosa* as antioxidants at various concentrations, compared to ascorbic acid, effectively inhibited DPPH free radicals. The highest antioxidant activity was observed in the ethyl acetate extract, achieving 85.5% inhibition at a concentration of 200 µg/ml. This percentage indicates a significant superiority over other crude extracts. The enhanced activity may be attributed to the high proton density in the ethyl acetate extract, resulting from the chemical synergy of its constituent compounds (Shafek et al., 2015; Teffo et al., 2010). This extract inhibits DPPH free radical activity by donating protons, converting them into stable radicals (Habibi et al., 2019). As shown in Table 5, the effectiveness of the extract increased with higher concentrations through enhanced activation. A similar result was reported by Ren et al. (2019), who noted a significant increase in DPPH radical scavenging activity with concentrations rising from 25 to 200 µg/mL, peaking at 91.7% at 300 µg/mL.

As mentioned, the hot water extracts of *D. Viscosa* leaves exhibited the lowest free radical scavenging activity across all concentrations. This may be due to a deficiency of protons in the aqueous extract, resulting from a lack of chemical compounds (Teffo et al., 2010). Furthermore, extraction was performed using a sequential solvent system, with the aqueous extract obtained after four solvents of varying polarities. This process facilitated the polarization of leaf chemical compounds based on their polarity, reducing the residual chemical compounds in the plant material. Consequently, the quantity of polarized compounds in the hot water extract was lower than in previous extracts. Teffo et al. (2010) investigated the antioxidant activity of compounds isolated from *D. Viscosa* leaves using the DPPH method and reported significant antioxidant activity. The antioxidant activity and total phenolic content of *D. Viscosa* leaf extracts were studied by Shoriqi et al. (2021), who reported significant differences among the solvents used. Phytochemical analyses further revealed that *D. Viscosa* extracts primarily contain phenolic and flavonoid compounds. These findings align with a recent report indicating that sequential solvent extraction depends on solvent polarity, with extraction efficiency closely tied to this property (Kanmani Bharathi and Prakash, 2025; Teffo et al., 2010). The high solubility of phenols in polar solvents results in higher concentrations in the extracts (Subramanian et al., 2023). The increased phenolic content with the application of HA or NPK fertilizers can be attributed to the synergistic effects of HA components with fertilization, which enhance phenol synthesis. Additionally, HA components promote glutamine synthesis, involving the binding of five gallic acid molecules to glucose, thereby increasing gallic acid production (Xu et al., 2021).

This study investigated the combined effects of HA and NPK fertilizers at various concentrations on the synthesis of phenolic acids, including quercetin, gallic acid, salicylic acid, para-hydroxybenzoic acid, and cinnamic acid. Our results revealed significant variations in phenolic acid content under the studied treatments. These outcomes are attributed to the influence of NPK and HA in enhancing photosynthesis rates and carbohydrate formation, which enter the glycolytic metabolism to produce triose phosphate (Shoriqi et al., 2023). These compounds are converted into shikimic acid via the shikimate biosynthetic pathway, from which most phenolic compounds in plants are derived as secondary metabolites. These findings align with those of Xu et al. (2021) in *Rosa rugosa*. However, high levels of fertilization can reduce the efficacy of these compounds as antioxidants and inhibit their activity.

Eleven compounds were identified in the organic layer (diethyl ether) of *D. Viscosa* leaf extracts using gas chromatography, as presented in Table 13. The most prevalent compounds identified were myrcene, eugenol, and alpha-terpineol, with relative peak areas of 43.431%, 20.122%, and 15.501%, respectively. These compounds include monoterpene oils with the chemical formulas C10H16 for myrcene, C10H18O2 for eugenol, and C10H18O for alpha-terpineol. Myrcene (7-methyl-3-methylene-1,6-octadiene) is used as a flavoring agent in the food, manufacturing, and beverage industries. Several studies have demonstrated its potential anticancer activity in in vitro models (Chawki et al., 2009). Alpha-terpineol exhibits notable antioxidant activity and can be produced naturally through fungal or bacterial biotransformation of limonene (Peshchke et al., 2009). Eugenol, also known as clove oil, is an aromatic oil extracted from cloves and is commonly used for treating toothache. These results are consistent with the essential oil profile of *D. Viscosa* leaves from Saudi Arabia (Makawi and Mousa, 1981). The findings highlight the diverse chemical profile of *D. Viscosa* essential oils, supporting their potential applications in pharmaceutical, cosmetic, and industrial fields.

Conclusion

This study investigated the effects of different concentrations of humic acid (HA) and NPK fertilizer on the growth, nitrogen, phosphorus, potassium content, and phenolic acids of the medicinal plant *Dodonaea viscosa*. Seedlings treated with HA (2 ml·L^{-1}) and NPK (0.5 g per pot) exhibited significantly enhanced growth and higher phytochemical content. Moreover, the combined HA and NPK treatment produced the greatest increases in plant height, leaf and branch number, as well as fresh and dry biomass, clearly demonstrating a synergistic effect between the two fertilizers. Phenolic content and antioxidant activity reached their highest recorded levels under this treatment, reflecting the high biological and medicinal potential of *D. viscosa*. These results were confirmed by both qualitative and quantitative assays, including total phenolic content and DPPH analysis. The study also highlighted the importance of ethyl acetate extract as a source of native phytochemicals and complementary antioxidants for use in traditional medicinal systems. Overall, these findings indicate that the optimized application of HA and NPK can simultaneously enhance plant growth and the production of valuable secondary metabolites, supporting its application in medicinal agriculture and traditional therapeutic practices.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declare no competing interests.

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Availability of data and material

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The author conducted the experiments in collaboration and wrote the manuscript.

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Figures

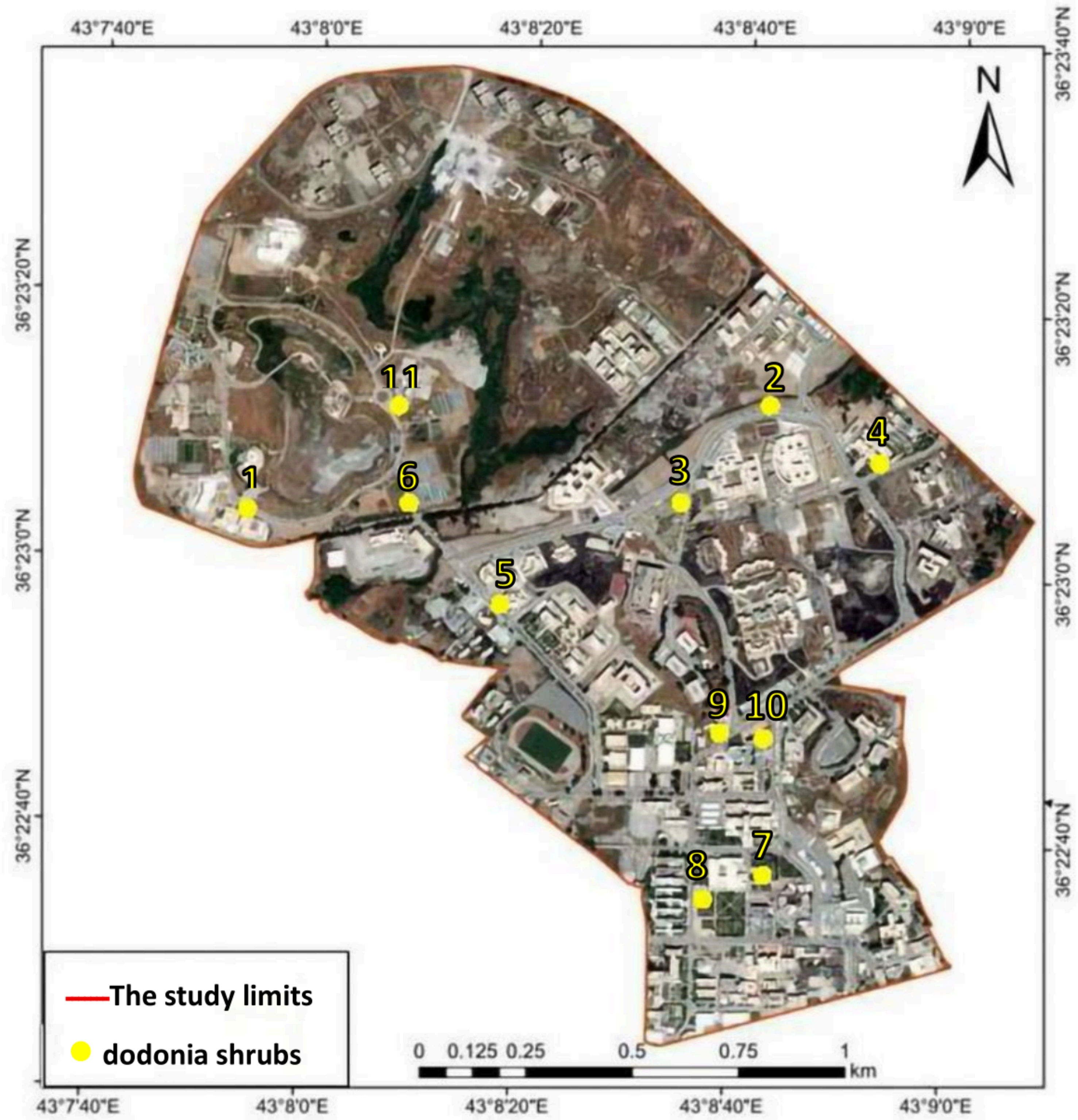

Figure 1

An air image of some administrative, field and marketing units of the University of Mosul Planted with dodonia using GIS

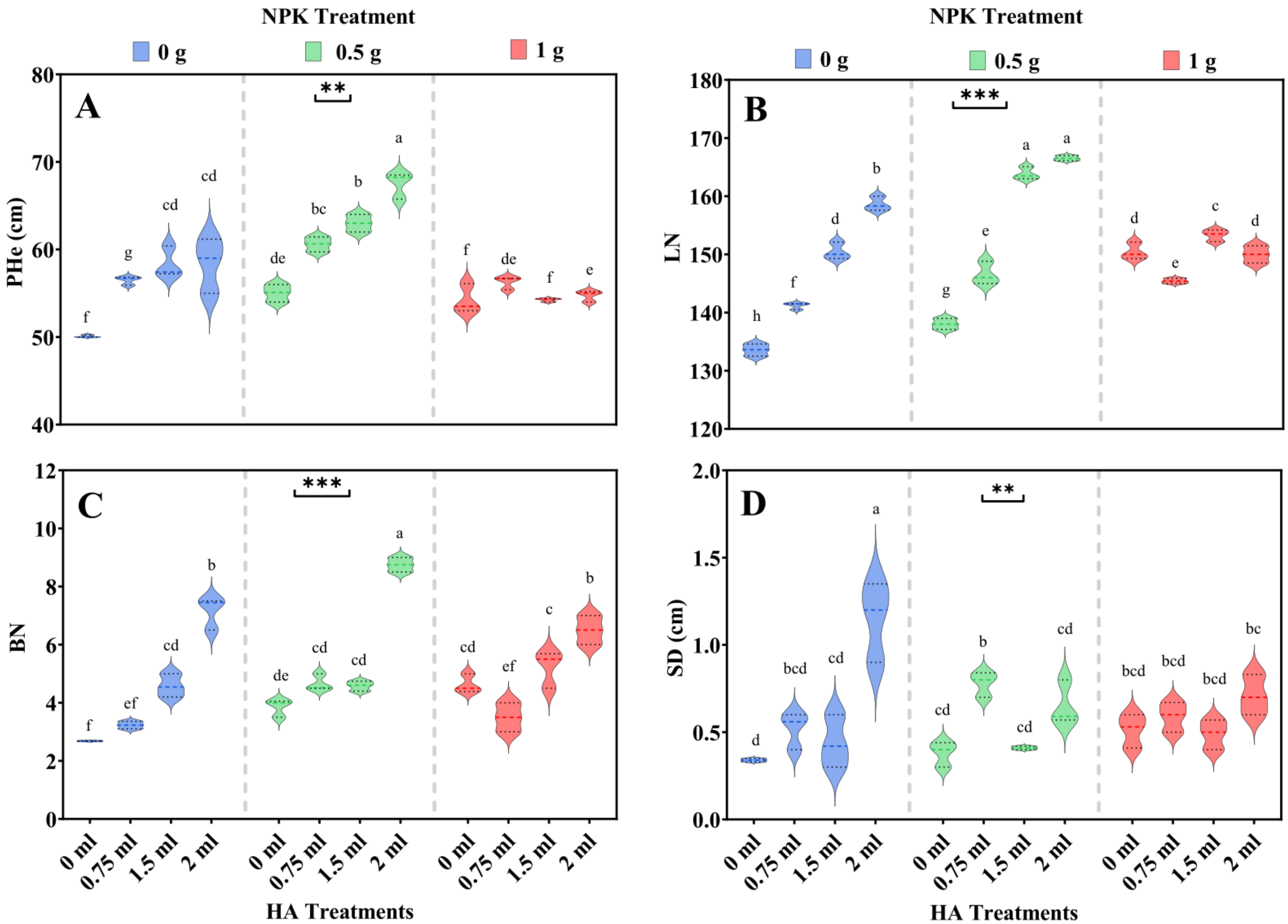

Figure 2

Effect of spraying with Humic acid at four levels (0, 0.75, 1.5, 2) ml L^{-1} and chemical (NPK 20:20:20) at three levels (0, 0.5, 1) g pot^{-1} in (A) plant height (PHe), (B) leaf number (LN), (C) branch number (BN) and (D) stem diameter (SD) of *Dodonaea Viscosa.*

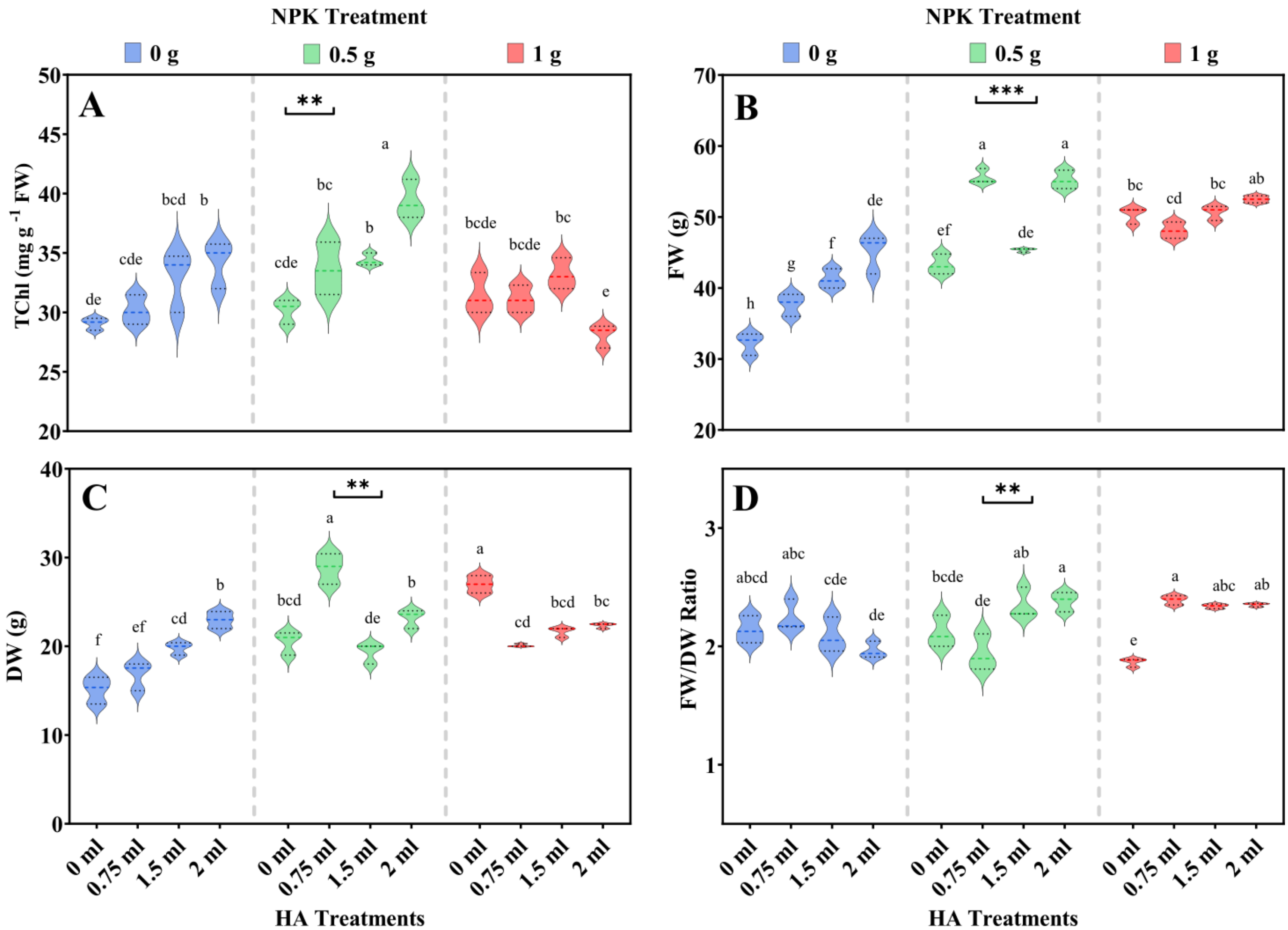

Figure 3

Effect of spraying with Humic acid at four levels (0, 0.75, 1.5, 2) ml L^{-1} and chemical (NPK 20:20:20) at three levels (0, 0.5, 1) g pot^{-1} in (A) total chlorophyll (TChl), (B) fresh weight (FW), (C) dry weight (DW) and (D) ratio of FW/DW in *Dodonaea Viscosa.*

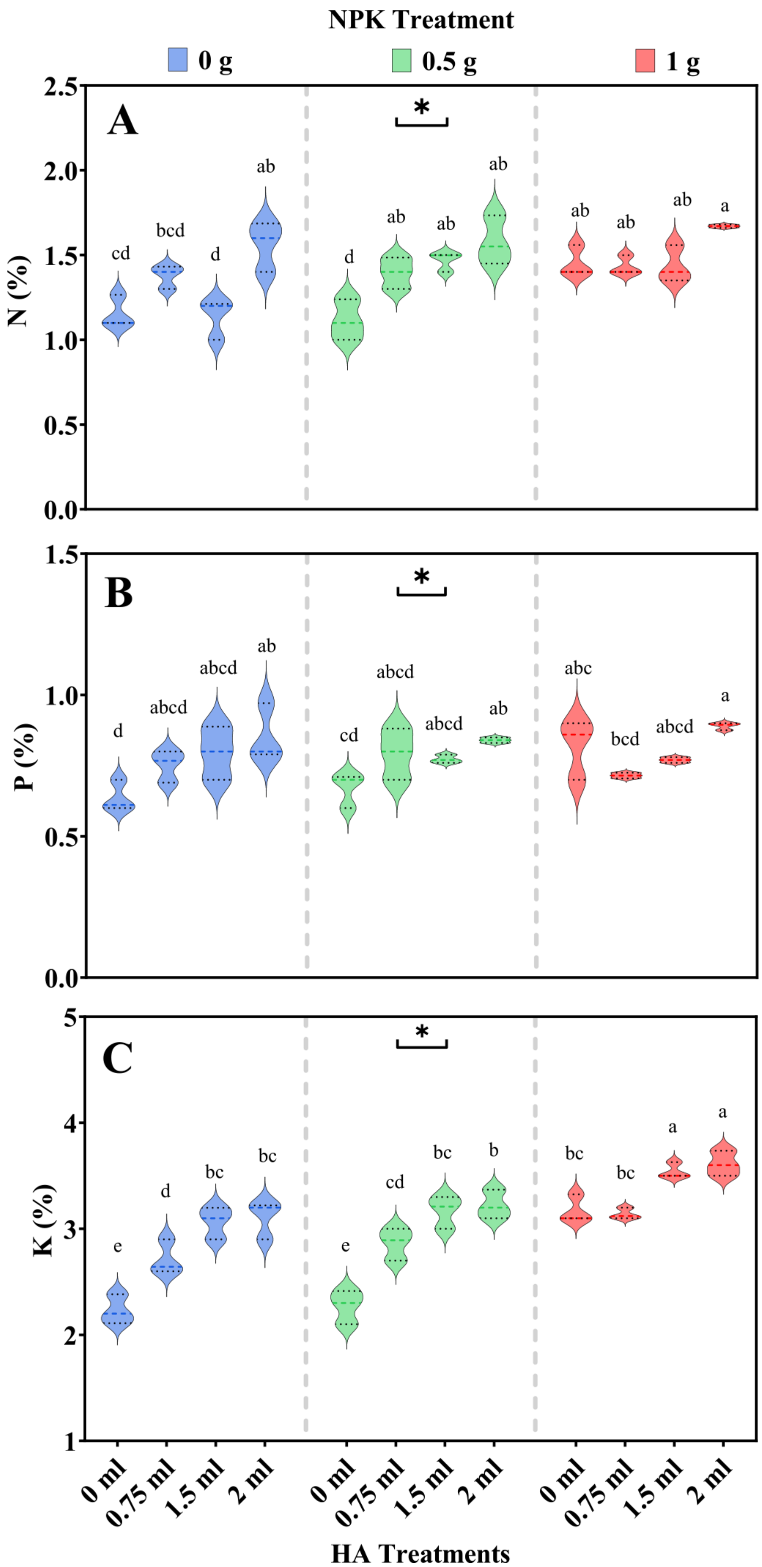

Figure 4

Effect of spraying with Humic acid at four levels (0, 0.75, 1.5, 2) ml L^{-1} and chemical (NPK 20:20:20) at three levels (0, 0.5, 1) g pot^{-1} in (A) nitrogen (N), (B) phosphor (P) and (C) potassium (K) of *Dodonaea Viscosa.*

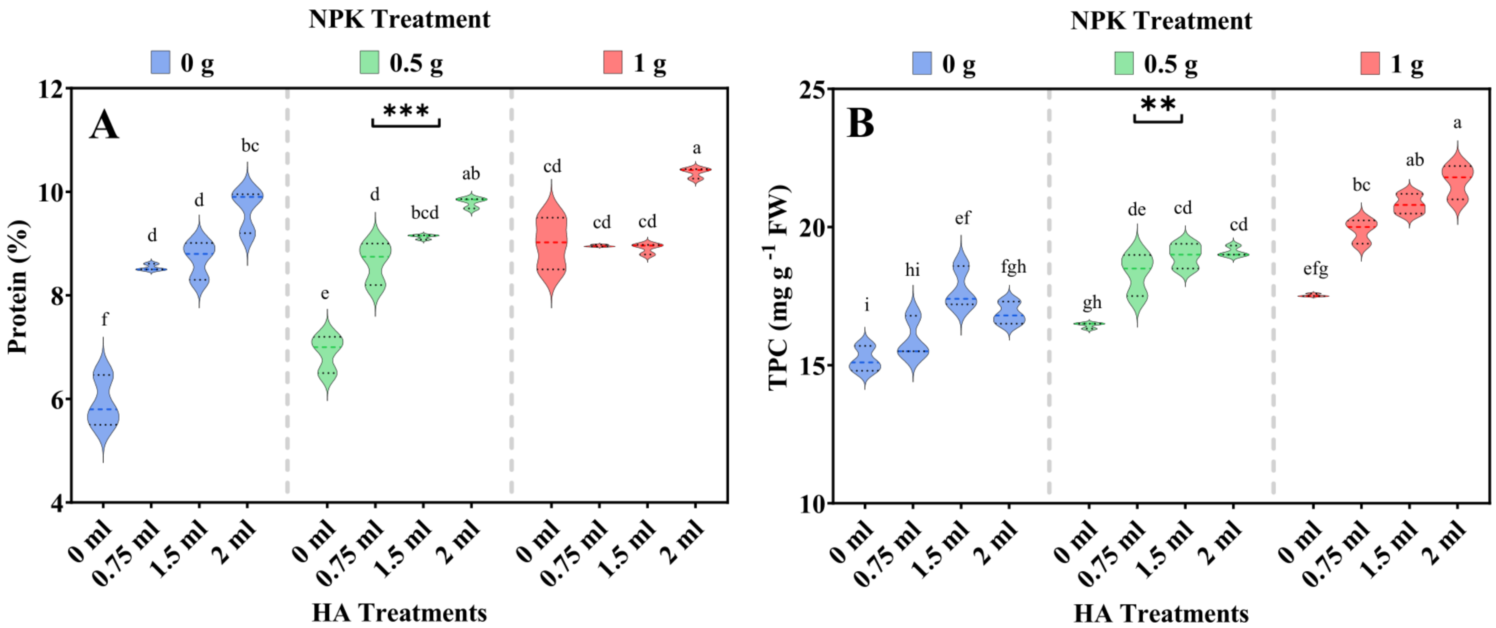

Figure 5

Effect of spraying with Humic acid at four levels (0, 0.75, 1.5, 2) ml L^{-1} and chemical (NPK 20:20:20) at three levels (0, 0.5, 1) g pot^{-1} in (A) protein and (C) total phenolic compounds (TPC) of *Dodonaea Viscosa.*

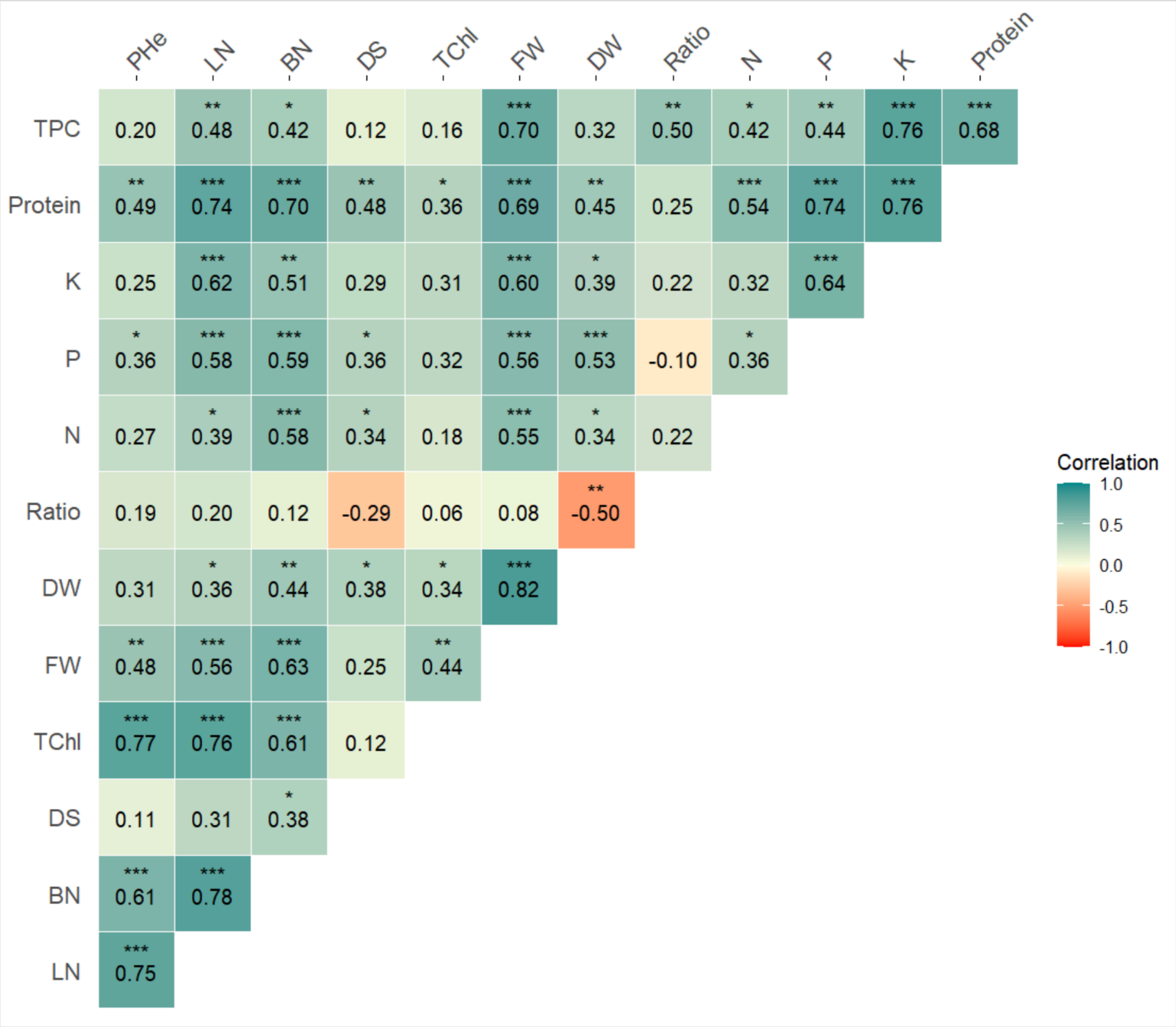

Figure 6

Correlation coefficients between the morphological and physiological traits of grapevine cultivars and rootstocks under SS and AA treatment. The color spectrum, from Salmon to dark cyan, represents highly negative to highly positive correlations. The numbers in the figure are the Pearson correlation coefficients between the indicators. Stars in the squares indicate the significance of correlations (*P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001). See Fig 2,3,4 and 5 for the definitions of the measured traits.

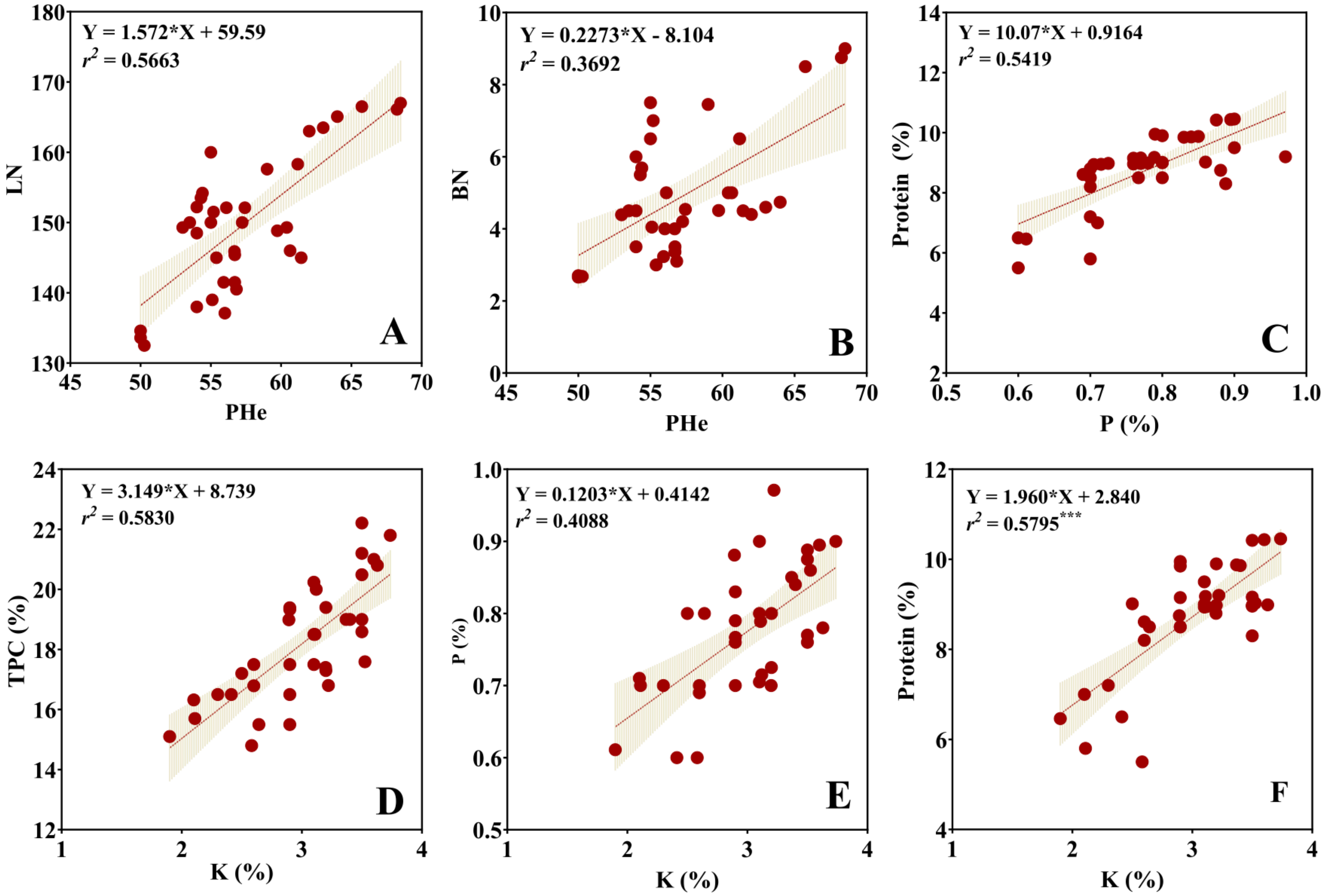

Figure 7

Reltionship between plant height (Phe) with (A) leaf number (LN) and (B) branch number (BN), and (C) phosphor (P) with protein, and potussiom (K) with (D) total phenolic compounds (TPC), (E) P and (F) protein. The correlation coefficients (r) of the relationships are given (P < 0.05).

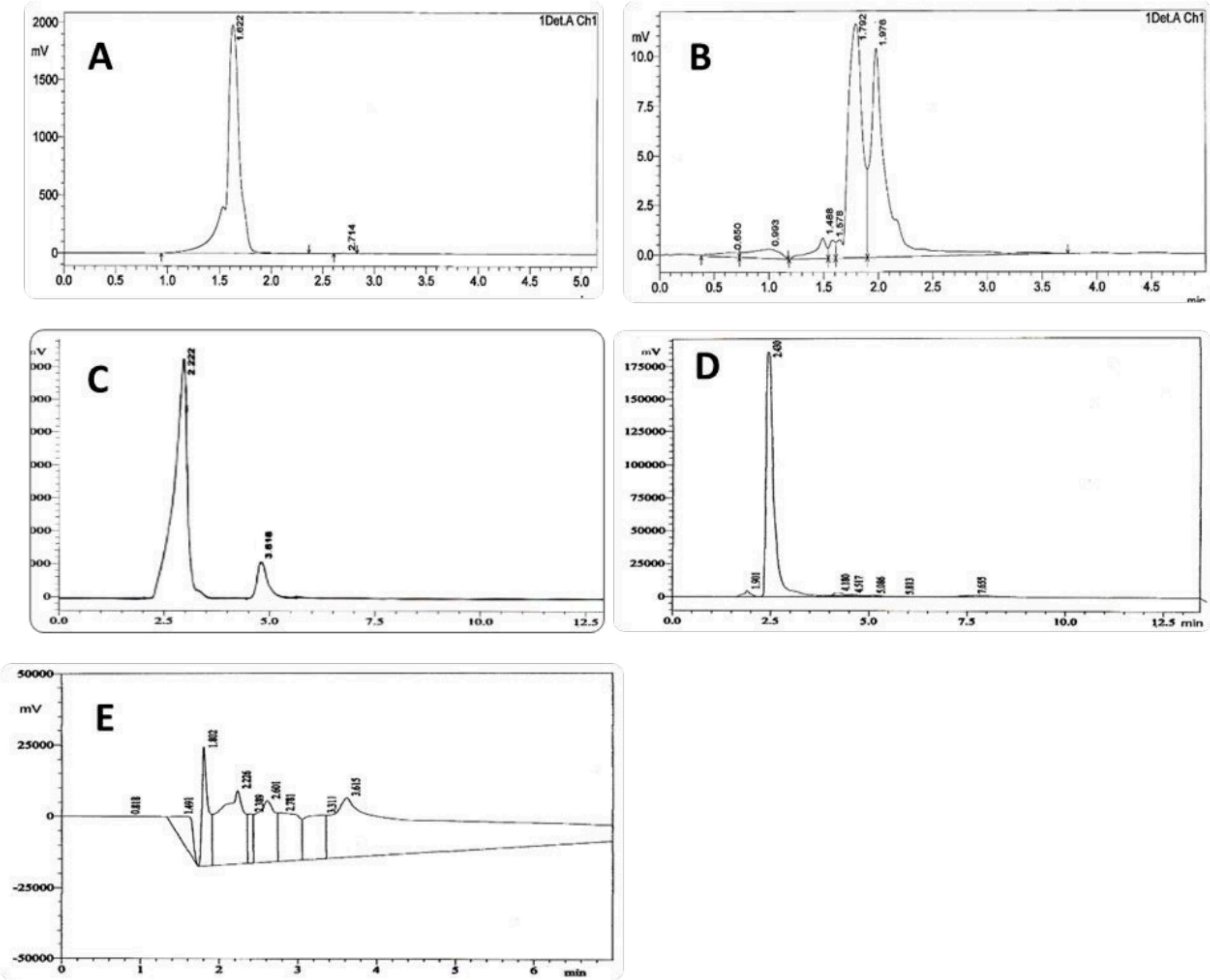

Figure 8

Chromatograms HPLC of standerd phenolic compounds. (A) Salicylic acid, (B) P-Hydroxy benzoic acid, (C) Cinnamic acid, (D) Gallic acid and (E) Quercetin acid

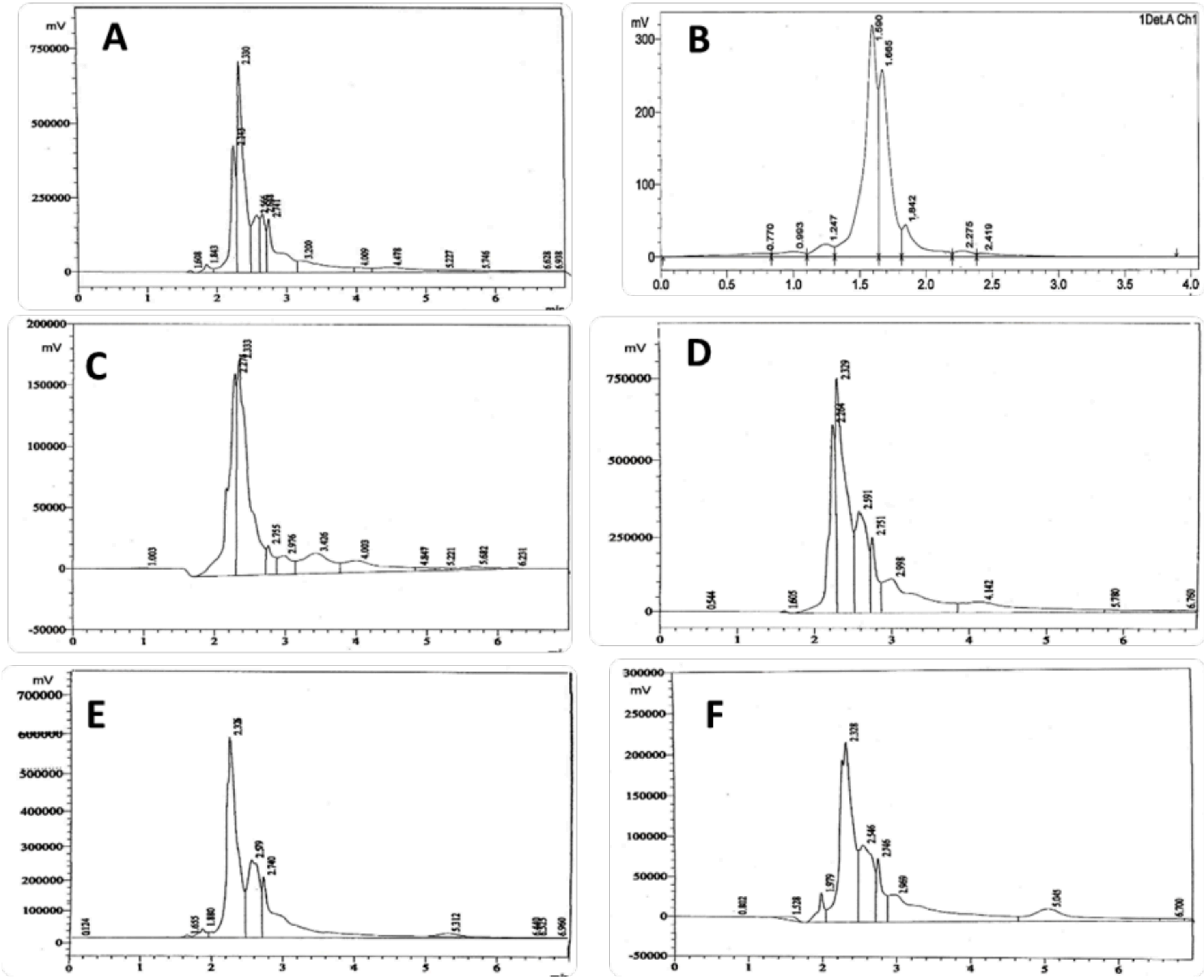

Figure 9

HPLC results showing the effects of treatments with humic acid (HA) and NPK: (A) 0.5 g pot-1 NPK, (B) 0.75 ml L-1 HA and 0.5 g pot-1 NPK, (C) 1.5 ml L-1 HA and 1 g pot-1 NPK, (D) 1.5 ml L-1 HA and 1 g pot-1 NPK, (E) 1.5 ml L-1 HA and 1 g pot-1 NPK, (F) 1.5 ml L-1 HA and 1 g pot-1 NPK

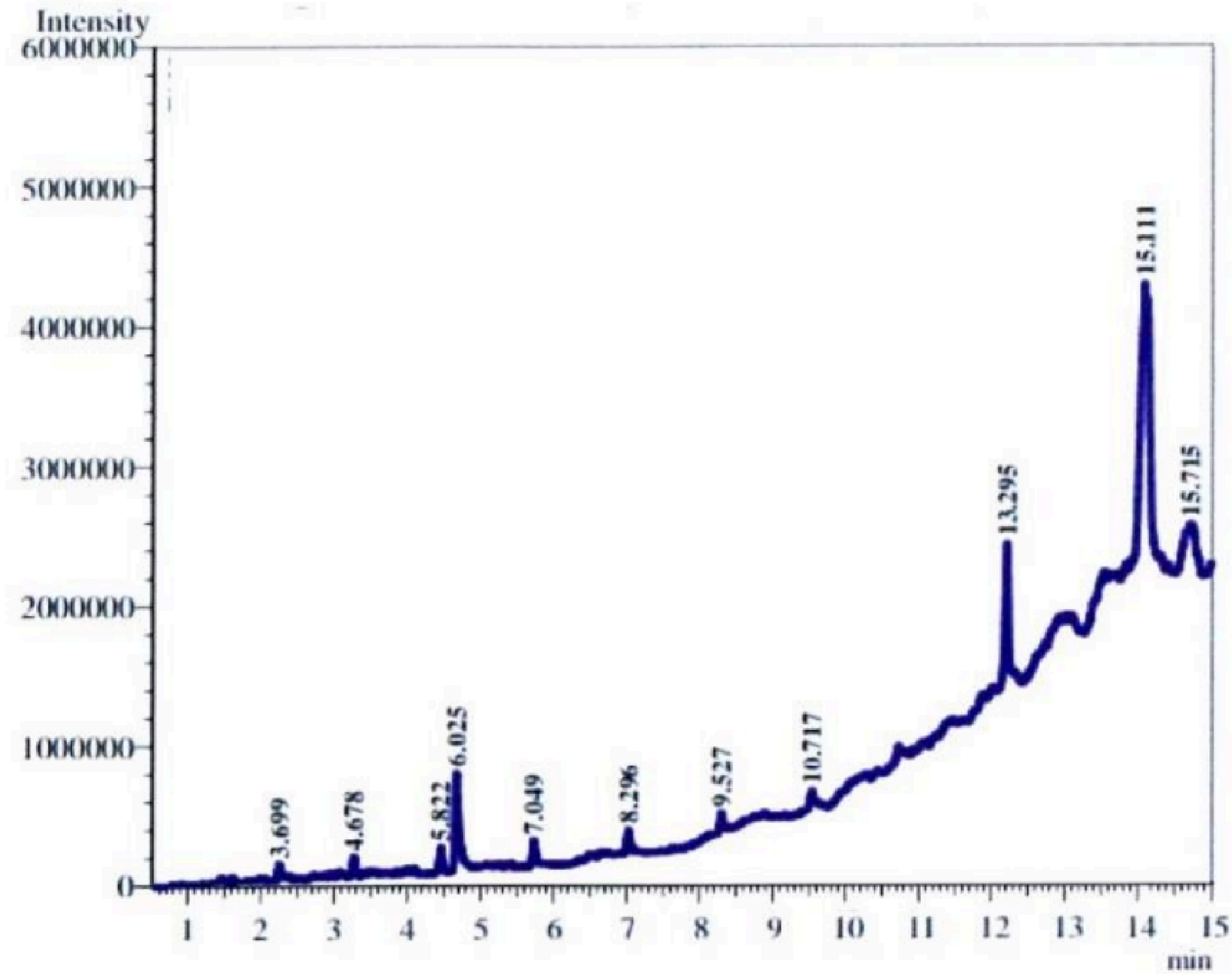

Figure 10

The percentage of area and retention time fer compounds shown by using GC-Ms device