

Yousif, Mohammed Dhary

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EFFECT OF DEFERENT LEVELS OF FERTILIZER 9DAP) IN CONTENT OF PLANT LEAVES FROM PHENOL COMPOUNDS AND (NPK)

MOHAMMED DHARY YOUSIF¹

Abstract: Carried out the experiment in the Republic of Iraq in the capital Baghdad and in the Dora district to study the effect of different levels of fertilizer (DAP) in content of plant leaves (*Calotropis procera*) in terms of phenolic compounds, as well as content (N, P, K), in pots where the soil used sandy silt collected from the shoulder of the Tigris River where contained soil 750g/ kg sand, 200 g/kg Clays, 50 g/ kg clay, average virtual soil density 1.1 Mega grams/ m³, the average electrical conductivity (Ec) 1.8 ds/m, the degree of interaction (pH) 7.2, the concentration of nitrogen-ready 20 mg/kg, soil and phosphorus ready mg /kg 15 and 145 ready-potassium mg /kg soil. the experiment included add four levels (100 . 200 . 300 . 400) kg / h of DAP fertilizer (18:46 N: P) to the publican seedlings which are Elected age of one year and using fully randomized design (CRD) and in three replications. in dated 10/15/2014. After the sample was taken leaves of the plant to assess the content of phenolic compounds (Gallic acid, Vanillic acid, Epicatechin, P-coumaric, Ferulic acid, Queretin-3-β-D- glucoside, Rutin) as well as total chlorophyll and (N, P, K). Results of statistical analysis showed the least significant difference (LSD) at a level of 5% higher than the level of fertilization 400 kg DAP /h on the rest of the levels followed by a level 300 and level 200. Where the increase in the rates of securities content of phenolic compounds, especially Epicatechin (48.72, 87.20, 137.18%) compared to the first level of 100 kg DAP / h sequentially with significant differences. The percentage increase in the stock of the content of p- coumaric (43.82, 85.39 and 92.13) % to the level of the second, third and fourth sequentially compared to the first level. As for (Ferulic acid, Queretin - 3β-D-glycoside, Rutin and Chlorophyll) in fourth level of 400 kg DAP /h has achieved the highest concentration content where the percentage increase (1050, 86, 164.81 and 40.47) % sequentially compared to the first level 100 kg DAP /h. Nitrogen, Phosphorus and Potassium, where the focus accounted for an increase of (80.99 and 155.00 and 65.38%) compared to the first level and sequentially. The results show that the plant () showed a steady and flexible response to the levels of fertilizer DAP, Increase the speed of absorption of key nutrients (N and P), to increase the readiness of those elements in the soil solution, increasing the shoot and root. Increase the efficiency of absorption of the major key elements (K, P, N) content and increase the stock of vehicles and phenolic acids and thus increase in the proportion of plant chlorophyll content.

Keywords: *Calotropis procera*, phenolic compounds, dap fertilizer (N, P, K).

JEL Classification: Q3

INTRODUCTION

(*Calotropis procera*) has been given several names. It is called pubic plant which is species of flowering plant. It is from the family Apocynaceae, a desert plant that spreads in North Africa, Tropical Africa, Western Asia, South Asia, and Indochina. It can be described as an evergreen tree with a height of 2-5 meters, having large branches and large leaves whose shape is oval between 5.7 cm - 15 cm. Its width is between 4.5 - 8.2 cm, with a sharp tip, and a light green color. The flowers are regular and have violet color. They are collected in coronal or inflorescence. The date of flowering is in May, June and July. The fruit is spherical or semi-elliptical, a large spongy apple color and shape. It is full of seeds bearing at its peak silky filaments known as fluff and all its tissues contain a liquid toxic substance with milky color, which is tart and extremely bitter.

The plant (*calotropis procera*) has a deep dense roots and it grows in areas of severe drought. Is a medicinal plant called *calotropis procera* of the family *Asclepiadaceae* (Parrotta, 2001). It is proposed to be on the list of plants that can be used to produce biofuels for its ability to produce an annual vegetable crop of 90 ton /ha (Al-Zahrani, 2002 and Boutraa, 2010 and Ibrahim, 2013). It is a plant that resists water and saline stresses. It is one of the promising plants that combats pollution due to its root system to absorb heavy elements such as Cd and Se without physiological damage (Al-Qahtani, 2012 and Al-Yemni et al., 2011). It is also an important source of pharmaceutical drug production (Silva et al., 2010). Its leaves can be a source of flavonoids and phenols such as, flavonoid quercetin, flavonoid glycosides, phenolic or polyphenol (Khasawneh et

¹Dr., Ministry of Higher Education and Scientific Research, Al-Karkh University of Science, E.mail: mdhj1965@yahoo.com, Mobile: +9647728131130

al. 2011, Murti et al., 2013). Phytochemicals are of great importance as they are natural sources of antioxidants. They are important for human health, food flavor and cosmetics, and are the most environmentally friendly and safest in consumption compared to their manufactured counterparts (Shrikumar & Ravi, 2007). The contribution of known plants to human health is mostly due to their phenolic compounds, mainly flavonoids and phenolic acids that act as antioxidants against ROS (Reactive Oxygen Species). ROS is a harmful oxidant of biomolecules such as, lipids, nucleic acids, proteins and carbohydrates. Fu and his colleagues (2011) found that there is a linear relationship between the amount of antioxidants and plant content of phenolic compounds in 50 medicinal plants. Multiple phenols have a role in reducing oxidation stress in plants and improving their resistance to diseases, especially viral ones. Oxidation and oxidative stress cause many diseases including rheumatism, cirrhosis, arteriosclerosis, diabetes and cancer (Ebadi, 2006).

Phenolic compounds are secondary plant products that are useful and important compared to other plant groups. They have a role in the production of various effective medical drugs (Pandey, 2013). (Harborne, 1989) has demonstrated that Phenolic or Polyphenol can be chemically defined as an aromatic substance known as Polyphenol (Hydroxyl Substituentases) including functional derivatives (esters, methyl and glycosides and others) (Harborne, 1989 or based on the number of phenolic rings and the structure of these rings).

Ammonium Diphosphate fertilizer (DAP) is an effective source of nitrogen, phosphorus and ammonium (IPNI, 2014). N and P are essential nutrients needed by the plant in large quantities for their important roles in building proteins, nucleic acids and energy (Mengel & Krikby, 1982). The quantities needed for each nutrient crop vary depending on the type of soil, the milieu in which the plant grows, the type of plant, its production capacity, as well as the surrounding environmental conditions. Ammonium Diphosphate fertilizer (DAP) (18 - 46 - 0) is one of the group of ammonium phosphate salts produced by the interaction of ammonia and phosphoric acid. It is used as a chemical fertilizer for plants, a source of nitrogen and phosphorus. It temporarily raises soil alkalinity, but after a period of time the treatment becomes more acidic due to Ammonium decomposition. Nitrogen is the main compound in the important substances that contribute to the structure of the plant as it forms 40-50% of the dry matter of the protoplasm. Therefore, *Calotropis procera* needs high amounts of nitrogen to complete the various growth processes. Some nitrogen substances move a lot inside the plant and this makes it able to reach to the developing peaks of the plant. That is, they move from the old tissues to the modern tissues, so the symptoms of the lack of nitrogen appear on the lower parts (old ones) while the upper parts (modern) are less affected. The symptoms of burning papers can be distinguished because of the lack of nitrogen element from the symptoms of leaf burning due to drought, which affected the parts of the plant, the upper and lower ones at one time.

Based on this, the study aims at achieving the following objectives:

- 1- Studying the effect of different levels of dap in the content of some phenolic compounds (Gallic acid, Epicatechin, Vanillic acid, p- coumaric, Ferulic acid, Quercetin-3- β - glucoside and Rutin) in the leaves of the *Calotropis procera*.
- 2- Studying the effect of levels of DAP.
- 3- Studying the effect of DAP levels in the total chlorophyll content in the leaves of the *Calotropis procera*.

MATERIALS AND METHODS

An experiment was carried out in a pot, each with a capacity of 20 kg with a sandy soil, where the ratio was used (750 g / kg of sand and 250 g / kg of soil). The average soil density was 1.1 mg / m³, the average electrical conductivity (EC) was 1.8 msi / m; the temperature (7.2 °C); the nitrogen concentration was 20 mg / kg soil; ready-made phosphorus 15 mg / kg; and ready-made potassium 145 mg / kg soil. The experiment included the addition of four levels of DAP (N: P) (18:46, 100: 200, 300: 400) kg DAP / ha, which were added in four equal increments (10, 20, 30 and

40%) of the total amount of fertilizer to seedlings after selected at the age of one year and 20 days between one addition and another since the date of the first increment. At the start of the experiment, the irrigation process was carried out whenever necessary, as it is a desert plant that tolerates the water stresses. The treatments were repeated three times, thus the number of replicates is 12.

Statistical Analysis

Design Random Complete (CRD) was used in the experiment. The results were analyzed with the least significant differences (LSD) and with a significant difference of 0.05 and with the use of the Iraqi compound mineral fertilizer 200 kg / e (10:18) N: P) for the experiment as a Starter. Evaluation of soil before planting was carried out according to the following methods in the analysis:

Pipette method: In the analysis of the size of the minutes and according to the method described (Day, 1965), the soil reactivity (pH) was estimated at 1: 1 using a PH-meter as described by (Jackson, 1958). Electrical conductivity (Ec) was measured in soil solution (1: 1) using Conductivity Bridge Electrical device (Jackson, 1958). Soil content of ready-made nitrogen was determined using the Microkjeldahl method (page, et al., 1982).

Soil content of phosphorus which is available in soil is ready to be absorbed by the plant was extracted by Olsen method using NaHCO_3 with 0.5M concentration. It was colorized with ammonium molybdenum and ascorbic acid and was estimated using spectrophotometer at 882 nm (page, 1982). Soil content of potassium which is available in soil is ready to be absorbed by the plant. 1 M of CaCl_2 was extracted and estimated using a Flame photometer (Martin & Sparks, 1983). The basis of the idea for the indirect method is to extraction of nitrogen from the soil by the KCl solution with a shake for half an hour then it is exposed to filtration then a certain size of the filtrate is put in a glass jug. The distillation process then starts with the presence of (Divarda alloy) and (magnesium acid). Ammonia is received in boric acid in the presence of a manual (green Bromariazole and methyl red) and it is then tied using the HCL known as strength.

On 15-10-2014, the leaf was taken from each treatment to estimate the nitrogen, phosphorus and potassium element. It was washed with distilled water and dried in the oven at 65 °c until the weight was stable. It was then crushed and placed in plastic cans for chemical analysis. The analysis was carried out after the procedure of wet acid (dissolving the acids that melt the crushed leaves and then disposing them in physical ways without affecting the ingredients to be studied). They were measured according to the methods which are referred to from (Hayens, 1980).

Total chlorophyll measurement: it was measured by the SPAD field chlorophyll measuring device (fresh leaf wash with water, of which 1 g is crushed in 5 ml of acetone 80. The extract is filtered by filter paper, isolated in a tube, and completes the size of the cylinder to 5 ml and zeros the device using a container tube on a Seaton 80%. The light density of the leaky is read by placing it in the cell of the spectrometer wavelength of 663 nanometer and 645 nanometer. The quantities of multiple phenolic compounds are estimated after being extracted from plant leaves using (Ethyl acitate) by the method of high-performance liquid technology HPLC (performance Liquid Chromatography) (Microgram /gram dry weight) in accordance with the following (Forsch, 1993). Data collected and analyzed by computer is according to the Gestates program for the lowest moral difference (LSD) at the level of confidence 0.05.

RESULTS AND DISCUSSION

1. **The content of the leaves of Gallic acid (%):** Table (1) shows there is a significant increase at a level of 300 kg/ha of 1.91 $\mu\text{g/g}$ dry weight as compared to the level of 100 kg/ha of 1.2691 $\mu\text{g/g}$ DW and a rate of increase of 51.6% while the highest content of the papers Gallic acid at the level of 400 kg/ha is 2.01 $\mu\text{g/g}$ dry weight as compared to the level 100 kg/ha and the increase reached 59.52% while there was a discrepancy between the first and second level as well as between level III and IV but it was not significant.
2. **Leaf content from vanillic acid:** Table (1) shows that the results of the statistical analysis increase with the level of fertilization, where the content of the leaves at 200 kg/ha is about 0.65 $\mu\text{g/g}$ dry

weight compared to the first level which is 0.28 µg/g dry weight with a significant discrepancy and an increase of 132% while the third level of 300 kg/ha reached about 0.95 µg/g dry weight compared to the first level which is 0.28 µg/g dry weight with a significant discrepancy and an increase of 239.28%. However, a higher impact at Level IV 400 kg/ha is 1.05 µg/g dry weight compared to level I which is 0.28 µg/g dry weight and by a significant discrepancy and an increase of 275%. Here, the effect of using higher levels of DAP fertilizer is illustrated by the increased volume of leaf content from phenolic compounds and this leads us to study higher levels to see how the results interact with them but within the limits of economic feasibility.

Table (1): The effect of adding different levels of dab fertilizer in the leaf content of multiple phenolic compounds microgram/g dry substance.

Studied djective	Ferulic acid	p-coumaric	Epicatechin	Vanillic acid	Gallic acid
Fertilizer levels					
100 kg/ha DAP	0.08	0.89	0.78	0.28	1.26
200 kg/ha DAP	0.48	1.28	1.16	0.65	1.61
300 kg/ha DAP	0.79	1.58	1.46	0.95	1.91
400 kg/ha DAP	0.92	1.71	1.85	1.05	2.01
L.S.D 0.05	0.061	0.11	0.126	0.061	0.454

- Plant leaf content from Epicatechin (%):** The results of the statistical analysis in Table (1) indicates the lowest significant difference (LSD) at the level of 0.05 which is significant, and the content of the leaves of the Epicatechin increased when we used the second level of DAB fertilizer (200 kg/ha) and the content of the leaves of the Epicatechin reached 1.16 µg/g (dry weight) compared to the first level of 0.78 µg/g (dry weight) and by an increase of 48.71% while the third level of 300 kg/e achieved a significant increase in the content of the leaves of the Epicatechin, which was 1.46 µg/g, relative to the first level and an increase of 87.17%. However, the fertilization effect reached a maximum in the content of the leaves of the Epicatechin and by a significant discrepancy of 1.85 µg/g (dry weight) compared to the third level and an increase of 137.18%.
- Plant leaf content from P-coumaric (%):** Table (1) shows that the P-coumaric is moving in the same direction to increase with all the levels of the total, with the content of the leaves estimated to be µg/g (1.28, 1.58 and 1.71) for the second, third, and fourth level in succession compared to the first level of 0.89. With significant variances, the rates of increase (43.82, 77.52 and 92.13) have been increased to the second, third and fourth levels in succession compared to the first level.
- The content of the leaves of Ferulic acid:** it can be noted from table (1) that the characteristic of Ferulic acid has been categorized to the level of significance where the highest content of the papers of Ferulic acid was affected at the highest level of fertilizer (DAP) which amounted to 0.92 µg/g dry weight where it was more significant than the rest of the levels including the first level of 0.08 microgram/g dry weight, while the second level reached 200 kg/h of 0.48 µg/gr from dry weight, with an increase of 5.00% compared to the first level of 100 kg/ha while the third level increase was 887.5%. The fourth level increased by 1050% compared to the first level.
- The content of the leaves of Queretin-3-β-D-glucoside:** With respect to the concentration of Queretin-3-β-D-glucoside in the leaves in table (2) it can be found that the highest level of DAP fertilizer 400 kg/ha has been given the highest concentration of 1.86 µg/gr from dry weight, and by a significant discrepancy of the first level 100 kg/h which reached 1.00 µg/gr from dry weight, with an increase of 86% compared to the first level. The effect of the second and third fertilization levels was in the same direction and the rate of increase was 41 and 74% in succession compared to the first level.

7. **Leaf content of Resin Substances:** It is noted from table (2) that all levels of fertilization increased the content of the leaves from the Resin substances and achieved qualitative and significant leaps, and values were (0.54, 0.94, 1.30 and 1.43) $\mu\text{g/gr}$ from dry weight and the increase was (79.6, 40.7 and 64.8)% compared to the first level of 100 kg DAP/ha with levels (200, 300 and 400 kg DAP/ha sequentially).
8. **Leaf content of total chlorophyll (%):** In the table (2) it can be found that fertilization with DAP fertilizer at different levels may stimulate plants to grow and this was evident in total chlorophyll and the percentages were (47.97, 56.16 and 59.00) % for the second, third and fourth levels in succession compared to the first level, which is 42.00%, with significant differences. The rate of increase was (14.2, 33.7 and 40.4%) in succession compared to the first level.

Table (2):The effect of adding different levels of dab fertilizer in the leaf content of multiple phenolic compounds $\mu\text{g/g}$ dry substance and chlorophyll SPAD and (N,P, K).

Properties Fertilizer levels	Queretin- 3- β -D- glucoside	(Resin materials)	T.Chlorophyll SPAD	N%	P%	K%
100kg/ha DAP	1.00	0.54	42.00	2.63	0.20	2.86
200 kg/ha DAP	1.41	0.97	47.96	3.73	0.29	3.30
300 kg/ha AP	1.74	1.30	56.16	4.26	0.40	3.73
400 kg/ha DAP	1.86	1.43	59.00	4.76	0.51	4.73
L.S.D 0.05	0.14	0.11	4.28	0.61	0.21	1.40

9. **Nitrogen concentration in the leaves (%):** The results of table (2) indicate that fertilization with chemical fertilizer has stimulated plants to take advantage of nitrogen which is available in soil and is ready to absorbed by the plant and this was clear as the concentration of nitrogen in the leaves was (3.73, 4.26 and 4.76)% for second, third and fourth level in succession compared to the first level which is 2.63%, with significant differences, while the hydrogen concentration between the second and third levels did not achieve any significant difference; the third and fourth levels did not produce any significant differences between them. The best nitrogen concentration in the leaves at the fourth level was 400 kg/h by an increase of 80.9% compared to the first level.
10. **Concentration of phosphorus in the leaves (%):**From the results of table (2) it can be noted that the addition of DAP at all levels has stimulated the plants to absorb phosphor and that is clear as the concentration of phosphorus was 0.40% for the third level compared to the first level which is 0.20% and with a significant difference and 100% increase ,while at the first and second levels, phosphorus did not achieve any significant differences; however, the concentration of phosphorus at the fourth level was 0.51% compared to the first level and by a significant increase of 155%.
11. **Potassium concentration in leaves (%):** The results of table (2) indicate that the levels of the DAP fertilizer have stimulated the plants to absorb potassium and this was evident at the highest level, as the concentration of potassium was (2.86, 3.30, 3.73 and 4.73)% to the first, second, third, and fourth level in succession and by an insignificant difference between first level and second level as well as between the third and the first as well as the third and second, while the concentration of potassium in the fourth level of 400 kg was (4.73%) compared to the first level and by a significant difference and an increase of 65.38%.The significant increase in leaf content from phenolic compounds and total chlorophyll, depending on the levels of added DAP fertilizer, is due to the containment of fertilizer on two essential elements, nitrogen and phosphorus, which played an important role in cell growth and division, protein building, energy industry sources, chlorophyll, various membranes, in addition stimulating roots growth by improving some characteristics of absorption mechanisms. Thus, this leads to the construction and formation of

phenolic compounds in addition to the role of the active DAP fertilizer in the improvement of nitrogen levels and phosphorus which are available in soil and are ready to be absorbed by the plant (Mengel and Krikby, 1982 and Havlin et al., 2005). The significant effect of the added DAP fertilizer in the leaf content of N, P, K% may be the ideal growth for the air parts and roots due to the levels of added DAP fertilizer that have led to the availability of elemental nitrogen, phosphorus and micronutrients by interception according to the good growth of the roots resulting in a moral increase in the absorption of N, P, K% (Havlin et al., 2005 and IPNI, 2014).

CONCLUSIONS

1. The results show that the (*Calotropis procera*) has shown a flexible and steady response to the levels of manure added in the leaf content of phenolic compounds, while the fourth level gave the best content in the leaves of phenolic compounds and major nutrients N, P, K.
2. The addition of a dab fertilizer (DAP) has increased the absorption speed of the main nutrients (N and p) necessary for protein construction due to the increased effectiveness of these elements in the soil solution, which are absorbed easily by the plant.
3. The manure (DAP) with the two main components (N and P) stimulates the increase of the total vegetativeness leading to a more efficient metabolism and to increase the total root leading to a more efficient absorption of nutrients from the soil and thereby increase the plant content of the chlorophyll ratio.
4. Increasing in the growth of the total root, increasing in the absorption efficiency of major elements (K, P, N), and increasing in the content of the leaves of compounds and phenolic acids which are an important source of production of compounds and drugs and a source of extensive innovative components such as: flavonoid glycosides, flavonoid Quercetin, Polyphenol or phenolic.
5. The symptoms of nitrogen deficiency begin on the lower (old) parts while the upper (modern) parts are less affected and here we can differentiate the symptoms of the combustion of the leaves due to the lack of nitrogen element where the lower leaves are affected only by the symptoms of combustion of the leaves due to dehydration which affects the upper and lower parts of the plant simultaneously.

Suggestions:

1. Conducting more complementary and in-depth experiments and studies with a view to optimize the levels of manure that should be used to achieve good economic results, given the plant's response in a steady increase when increasing the fertilizer dose for the four levels used in this experiment.
2. Due to its studies and research that are conducted by (al-Qahtani, 2012, Al-Yemni and others, 2011) regarding the ability of the plant to withstand the water and saline stresses and the ability of the total of root group to absorb the heavy elements of the soil such as Cd and Se without causing physiological damage to the roots, it is encouraging for the purpose of continuing studies on this subject because it has an impact in combating pollution and reducing the risks and harms of these elements in the soil, where it is considered a promising plant in this field.
3. Interest in the studies of this plant for its richness and large stock of many drug-producing compounds and a source of extensive innovative components such as, flavonoid glycosides and flavonoid quercetin.
4. Paying attention to the feasibility study of the production of biofuels from this plant due to its ability to produce an annual crop of (vegetative portion) by 90 ton/ha.



Figure (1): shows the whole plant is in the form of a tree height of 2-5 which has many branches and shows the large fleshy leaves.



Figure (2): shows the regular violets form separate top or axillary flowers.



Figure (3): shows the fruits that are open and appear full of seeds that carry filaments at their top silk known as fuzz.



Figure (4) shows a liquid white substance with a bitter taste called Calotropis procera.



Figure (5) shows the fruits before opening it having a spherical shape that is close to the oval shape. It is a spongy having a color like the color of an apple and its shape is also like the shape of an apple. It is full of seeds.



Figure (6): shows a flower at the beginning of its opening and a fruit in a floral axillary flower which indicates the size of the large leaves and fruits.

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