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Effect of DAP (P21%) Fertilizer on Total *Saliva officinalis* Flavonoid Content

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Abstract

The effect of (Di-Ammonium Phosphate DAP) (P21%), as plant fertilizer on content of the *Saliva officinalis.* Total flavonoid as an important active constituent was investigated in the present study. Three samples (F1, F2, and F3) of *Saliva officinalis* were grown in soil treated with DAP (100, 200, and 300) kg/hector respectively. Another two samples were studied: F0 as a plant sample grown in soil untreated with fertilizer and F4 as wild type obtained from market. Total flavonoids were estimated Quantitatively by Rutin standard curve and qualitatively by TLC method in corresponding to standard flavonoids. Results showed that different concentrations of DAP treated soil affected flavonoid contained of the Iraqi cultivated *Saliva officinalis* in different manner. The lowest flavonoid contained was untreated samples (F0), and the best result was obtained from plant in soil treated with 200kg/H of DAP(f2) when the soil treated with DAP plant fertilizer in concentration (200kg/H) F2 sample.

Keywords: Total flavonoids, Saliva officinalis, DAP21%, Plant fertilizer, TLC.

تأثير السماد ثنائي فوسفات الامونيوم P%21 على محتوى الفلافونويدات الكلية لنبات المرمية

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الخلاصة

تأثير السماد ثنائي فوسفات الامونيوم(DAP) ذي التركيز 21% على محتوى الفلافونويدات الكلية لنبات المرمية كأحد اهم المكونات الفعالة في النبات. أذ ستخدمت ثلاث نماذج من النبات((F3 F2, F1) تمثل ثلاث معاملات للتربة بمادة DAP وبتراكيز (100 و200 و 300) كغم \ هكتار على التوالي . كما ان الدراسة شملت نموذجين اخرين هما F0 الذي يمثل نبات المرمية مزروع بتربة غير معاملة بالسماد والنموذج F4 الذي يمثل نبات المرمية البري والذي تم الحصول عليه من الاسواق المحلية. أن الفلافونويد الكلية في النبات تم احتسابها كميا عن طريق المنحنى القياسي للمادة القياسية، ونوعيا باستخدام تقنية الفصل الملون بالطبقة الرقيقة وبالمقارنة مع فلافونويدات قياسية في هذه الدراسة. أظهرت النتائج تباين نسب وكميات الفلافونويدات الكلية بتباين الكمية المضافة من سماد DAP وبانماط متباينة بالنسبة لنبات المرمية المزروع في العراق. فقد كان اقل محتوى للفلافونويدات الكلية في النموذج غير المعامل بالسماد F0. أما أفضل النتائج فكان للنموذج F2 الذي يمثل معاملة التربة (التركيز 200كغم/هكتار من سمادDAP).

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Introduction

There are areas in world with nutrient deficiency and other areas of nutrient excess. Fertilizers are responsible for over half of global food production. Managing mineral plant nutrients requires careful application of science and skill to meet production, environmental, and social goals. A plant's normal growth and function depend on relatively high intracellular water content [1]. A plant-based diet – focusing mainly on vegetables, fruits, medicinal plants and whole grains – has become one of the most important guidelines for lowering the risk of human diseases. Vegetable plants and human being have unique relationship since time immemorial and they are play a vital role in the human life. People should consume several hundred grams of plant-based diet a day since it is a good source of nutrients and dietary fiber. Therefore, need to improve the nutritive value of the final products of vegetables plant. The important contributions of the nineteenth century, experimental plant physiology to agriculture was discovery that soil fertility and crop yields could be increased by adding several nutrients to the soil [2]. Di-ammonium phosphate (DAP) is the world's most widely used phosphorus (P) fertilizer. It is made from two common constituents' phosphoric acid and ammonia in the fertilizer and it is popular because of its relatively high nutrient content with its excellent physical properties [2]. Secondary metabolites from medicinal plants having a great attention and desired aromatic with therapeutic qualities, providing source material for the perfume and chemical industries. Among these plants, Sage (*Salvia officinalis*), is aromatic plant belonging to the Lamiaceae family, which is a perennial plants and native to Mediterranean area [3]. Sage is one of the oldest medicinal plants and has a great economic and industrial importance and most popular aromatic medicinal herbs used for medicinal purposes [3,4]. Sage was used for many ailments, including inflammation of the mouth and throe [5] in traditional medicine,. Leaves of sage were used to relive headache, flatulence, toothache, abdominal pain. Additionally, sage was also used traditionally in food preparation, flavoring agents in perfumery and cosmetics [5,6]. On the other hand, Sage are very rich in phenolic compounds, such as flavonoids, phenolic acids and phenolic diterpenes that possess high antioxidant activities [6,7]. Phenolic compounds are secondary metabolites, naturally present in all plant materials such as leaf, and shoot [8,9]. Phenolic compounds are antioxidants with redox properties, which allow them to act as reducing reagents, hydrogen donators, and singlet oxygen quenchers. They including flavonoids that have been reported to accelerate wound healing activity [10,11]. Antioxidants were usually employed in industry as product additives and in food processing and preservation to prevent undesirable changes due to oxidation an important deterioration process for oil and fats [12]. Moreover, antioxidants are important because they have the ability of protecting organisms from damage caused by free radical-induced oxidative stress [13], and can protect us against major diseases such as coronary heart disease and cancer in human [14]. Flavonoids and phenolic compounds exert multiple biological effects such as antioxidant, free radical scavenging and anti-inflammatory properties [15]. Oxidative damage in the human body plays an important causative role in disease initiation and progression [16]. At present, the increase on the demand for natural bioactive compounds that can be used as functional compounds for the food industry has led sources of plant fertilizer soil treated conditions that permits a good control of plant growth and development, and is currently in practice all over the world [17]. In this research, *S. officinalis* was cultivated as an importation medicinal plant especially nowadays due to different uses such as pharmaceutical, sanitary, cosmetic, and agricultural and food industries over the entire world [18]. The comparison of the essential oils [19] and total flavonoids contents of sage plants under soil DAP treated conditions was fewer reported available.

Therefore the aims of study were to investigate the yield of total flavonoids compositions of *S. officinalis* L. qualitatively and quantitatively under normal untreated soil condition and DAP(21%) treated soil conditions in an attempt to contribute to use of these alternative products and natural antioxidant agent for food and medicinal uses.

Material and Method

Soil treatment and sample collection

The following ***Saliva officinalis*** plant samples were used in the work:

F0: ***Saliva officinalis* plant** sample grown in DAP Free treated soil (as a control).

F1: ***Saliva officinalis*** plant sample grown in DAP100kg/h treated soil.

F2: ***Saliva officinalis*** plant sample grown in DAP200kg/h treated soil.

F3: ***Saliva officinalis* plant** sample grown in DAP300Kg/h treated soil.
F4: ***Saliva officinalis* plant** sample grown in DAP free treated soil as an old wild type plant obtained from local markets.

The procedure for soil treatment with different concentration of DAP was applied as in (Hussein,2015) study [19]. All collected samples were dried, weighted, powdered and applied for Volatile Oil estimation by Clevenger hydro-distillatory apparatus.

Total Flavonoids Extraction

All samples from the previous step were reflected for 8 hr using 200 ml of 2M HCl solution. The filtrate was cooled and transferred to a separator funnel. The aglycon moiety was extracted by 50 ml ethyl acetate 3 times each. The collected ethyl acetate layers for all samples were washed with distilled water to get rid of the excess acid then evaporated to dryness by rotary evaporator at 40 ºC.The dried residue for each sample were re-dissolved in 30 ml 50% ethanol [20].

Quantitative Estimation of total flavonoids

Rutin standard stock solution was prepared (1mg/ml in 50% ethanol), from which serial dilutions were made to get Rutin standard solutions with concentration of (0.01, 0.1, 0.2, 0.25 and 0.5) mg/ml in 50% ethanol. Amount of 1ml was transferred from each standard solution and from each extracted Flavonoid sample into a glass tubes separately, then 0.75 ml of 5% sodium nitrite solution was added and mixed well to be left to stand at room temperature for 5 minutes. To all tubes 1.5 ml of 10% $AlCl_3$ in 50% ethanol was added, shacked well and left to stand at room temperature for another 5 minutes. At last 5ml of 1N NaOH solution was added to all tubes. The absorbance was read at 510 nm, and a standard curve was plotted between each concentration and the absorbance, then the amount of total flavonoid in each sample was calculated as Rutin from the equation of straight line that obtained from the plotted curve [21].

Qualitative estimation of total flavonoids

Thin-layer chromatography (TLC) is a very commonly used technique because it is simple and cheap. For determining total flavonoids for each sample the following solvent mobile phase was used: Toluene: Ethyl acetate: Glacial acetic acid (36:12:5). Stander solutions was prepared 0.1 mg/ml in 50%ethanol from rutin, kaempferol, quercetin, luteolin, then by using one spot of each extracted plant Flavonoid and from each standard solutions, a thin layer chromatography was performed on silica gel $G_{f\ 254}$ aluminum plates which activated at 100 °C for 30 minutes in an oven and cooled at room temperature before use [22].

Results and Discussion

Determination of total flavonoids

Quantitative assay

The absorbance of the spectrophotometric analysis for rutin Standard Solutions at 510nm was shown in the Table-1.

Table 1- Spectrophotometric reading of rutin standard solutions at 510nm.

Concentration(mg/ml)	Absorbance (at 510nm)
0	**0.00**
0.01	**0.006**
0.1	**0.062**
0.2	**0.172**
0.25	**0.266**
0.5	**0.55**
1.00	**1.263**

Concentration calculation

The plotting process between varying standard rutin concentrations and the equation of a straight line is shown in Figure-1.

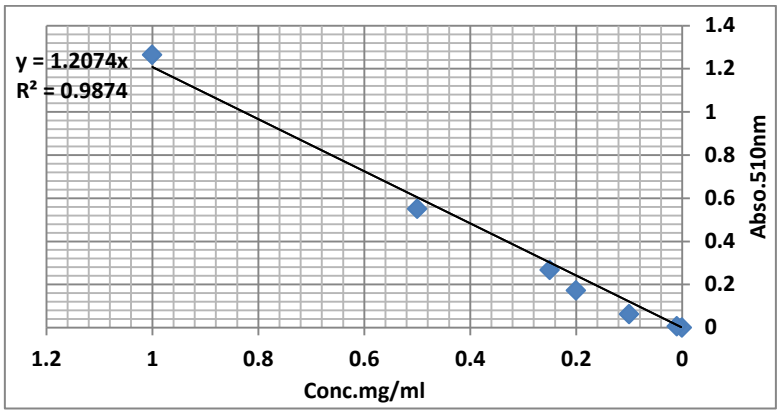

Figure 1- Standard curve for rutin as determined spectrophotometrically at 510 nm.

Y=1.207X
F1 absorption= 1.112-----Diluted ten times---X=9.213mg/ml X30=276.4 mg/SampleF1 =2.128 g%.
F2:1.552/1.207=1.29 X10-----X30=385.7 mg/sample F2 =2.3g %.
F3: 1.141/1.207= 0.9453 X 10=9.453 mg/ml X30=283.6 mg/sample F3 1.6 g%.
F4: 1.448/1.207=1.19966 X10=11.9967 mg/ml X 30=360 mg/sample F4 1.87 g%.
C0:1.570/1.207=1.3X10=13 mg/ml X30=390.223 mg/sample C0 1.9 g%.
F0:1.179/1.207=0.977X10 X30=293 mg/sample F0 =1.99 g%.

Table 2- Comparison of total flavonoid contents between different *S. officinalis* treated and untreated samples with DAP (21%) fertilizer.

Batch name	Weight(g)	% Total Flavonoid g/100g	DAP kg/H
F0	**14.73**	**1.99%**	**No**
F1	**12.99**	**1.128%**	**100**
F2	**16.85**	**2.3%**	**200**
F3	**17.83**	**1.6%**	**300**
F4	**19.24**	**1.87%**	**Wild plant sample**

Qualitative assay

Saliva officinalis leaves are rich with different flavonoids among them: Rutin (R), Kaempferol (K), Quercetin (Q), and Luteolin (L) as shown in Figure-2 in corresponding to standards.

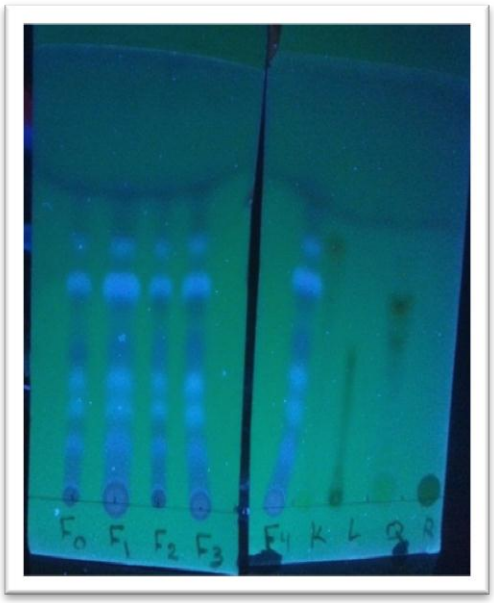

Figure 2- TLC Chromatogram for total flavonoids of all samples(F0, F1, F2, F3, F4) corresponding to standard solutions(Rutin=R, Quercetin=Q, Luteolin=L, and Kaempferol= K).

As shown in Table-2 a different results showed between all samples especially for the treated soil samples with DAP fertilizer. The control sample (F0) for *S.officinal* flavonoids yields 1.99% total flavonoids, while F1 samples appeared to give the lowest flavonoid contents(1.128%). At the contrast F2 was the greater total flavonoid content (2.3%). Total flavonoids were decreased to (1.6%) as there was an increasing in DAP concentration as in F3 sample. At last the wild type showed a total flavonoid content of 1.87%.

These results clearly demonstrate that the soil that treated with specific concentration from DAP (21%) plant fertilizer may affect the synthesis of one major constituent than other. For example F1 sample that treated with 100Kg/H DAP yielded greatest amount of volatile oil [19] and lowest content of total flavonoids thus because the concentration of fertilizer might affected soil pH and lead to change in active constituents pathway [23]. A notable property of DAP is the alkaline pH that develops around the dissolving granule. As ammonium is released from dissolving DAP granules, volatile ammonia can be harmful to seedlings and plant roots in immediate proximity. This potential damage is more common when the soil pH is greater than 7, a condition that commonly exists around the dissolving DAP granule. To prevent the possibility of seedling damage, care should be taken to avoid placing high concentrations of DAP near germinating seeds. The ammonium present in DAP is an excellent Nitrogen source and will be gradually converted to nitrate by soil bacteria, resulting in a subsequent drop in pH. Therefore, the rise in soil pH surrounding DAP granules is a temporary effect. This initial rise in soil pH neighboring DAP can influence the micro-site reactions of phosphate and soil organic matter, and this may explain the differences in volatile and total flavonoid content. Moreover the intensity of each flavonoid spot in Figure -2 for F0 differs from the intensity of identical flavonoid spots for sample F1, F2, F3 and so on which mean that addition of different concentration of DAP fertilizer will affect not only content of volatile oil or total amount of flavonoids, but also the type of the flavonoid the plant that will produce.

In one study showed that active components content in plant was depend on fertilizer type and could be tracked by different concentrations. In orchards, phenolics and antioxidant activity of jujubes could be manipulated through fertilizer management.

There are differences in the initial chemical reaction between various commercial P fertilizers in soil, but these dissimilarities become minor over time (within weeks or months) and are minimal as far as plant nutrition is concerned. Most field comparisons between DAP and monoammonium phosphate (MAP) show only minor or no differences in plant growth and yield due to Phosphate source with proper management.

In conclusion, this study provides new knowledge for effect cultivation methods with combination environmental conditions on chemical compositions essential oils and flavonoids for sage plant. To produce the best yield of them with a good concentration, it is necessary to combine suitable cultivation methods and fertilizer concentration that optimized the production of these compounds in the plant. Flavonoids and Essential oil composition for open field and ported soilless conditions is very variable, it is important that cultivation methods be optimized for some particular compound than others.

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