

## Effect of Different levels of Phosphorus Fertilizer on oil yield and their derivatives of three varieties of Safflower (*Carthamus tinctorius* L.)

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### ABSTRACT

Due to the important of Safflower oil in edible, industrial and medicine, this study was carried out under the rain-fed conditions at Experimental Farm of the College of Agricultural Sciences, University of Sulaimani at Bakrajo, during the winter season of 2017-2018, to study the effect of three varieties, namely (Zaafarani, Aidn and Al-Shamia), phosphorus applications (0, 50, and 100 Kg ha<sup>-1</sup>), and their interactions on oil yield, fatty acid compositions and some phenolic acid contents. Using Randomized Complete Block Design (RCBD) with split plot arrangement, with three replications, the main plots were allocated for the varieties and conducted in Randomized Complete Block Design (RCBD) with three replications, while the phosphorus levels implemented in sub-plots. Comparisons between means were carried out by the least significant difference (L.S.D) at 0.05 and 0.01 levels of significance. The results of this investigation confirm that the oil yield of the varieties ranged between 490-540 Kg ha<sup>-1</sup>, variety Al-Shamia produced the highest oil yield, and exceeded by approximately 50 Kg ha<sup>-1</sup> when compared to the lowest oil yield gained by Aidn. Al-Shamia recorded the highest significant concentrations of stearic, linolenic, linoleic, and oleic acids, while regarding to the phenolic contents the caffeic, ellagic, and p-hydroxybenzoic acids detected in more concentrations in Zaafarani compared to other two varieties, among the phenolics only the gallic acid detected with higher concentration in Al-Shamia followed by Aidn variety. The application of higher level 100 Kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> showed increasing in oil yield significantly compared to the lower levels 50, and 0 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, moreover it exceeded significantly the stearic, linolenic, linoleic and oleic acids compositions, but the higher levels of P<sub>2</sub>O<sub>5</sub> only increase the content of caffeic and gallic acids, while decreased the content of ellagic and p-hydroxybenzoic acid. The interactions response of the varieties to P<sub>2</sub>O<sub>5</sub> applications was not significant for the most traits of the study except for stearic acid composition, and ellagic, gallic, and p-hydroxybenzoic acids content.

**Key words:** Safflower varieties, Phosphorus Application, oil yield, fatty acid, and phenolic acids.

تأثير أضافة مستويات السماد الفوسفاتي في حاصل الزيت و مشتقاته لثلاث اصناف من

*Carthamus tinctorius* L. العصفور

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## الخلاصة

تم إجراء التجربة لدراسة تأثير التراكيز المختلفة من السماد الفوسفاتي على ثلاث اصناف من العنبر وتأثير تداخلهما على حاصل الزيت وتركيبات الأحماض الدهنية وبعض محتويات حمض الفينول. أجريت هذه الدراسة في ظل الظروف الديمية في محطة بحوث بركو- كلية العلوم الزراعية- جامعة السليمانية خلال الموسم الزراعي الشتوي 2017-2018، لدراسة تأثير مستويات اضافة السماد الفوسفاتي على ثلاث اصناف من العنبر وتأثير تداخلهما على حاصل الزيت وتركيبات الأحماض الدهنية وبعض محتويات حمض الفينول. نفذت التجربة باستخدام تصميم القطاعات الكاملة المعشاة وبترتيب القطع المنشقة، وبثلاث مكررات اذ شملت الألواح الرئيسة للتجربة الاصناف الثلاث للعنبر (الزعراني، ايدن، الشامي) بينما شملت الألواح الثانوية على مستويات السماد الفوسفاتي (0، 50، 100) كغم هكتار<sup>-1</sup> تم إجراء المقارنات بين متوسطات المعايير أعلاه باستخدام أقل فرق معنوي (LSD) عند مستوى 5% و 1%.

بينت نتائج هذه الدراسة أن حاصل الزيت للأصناف تراوح بين 490-540 كغم هكتار<sup>-1</sup>، وأن صنف الشامي أنتج أعلى حاصل زيت وفاق حوالي 50 كغم هكتار<sup>-1</sup> عند مقارنته بأقل حاصل زيت حصل عليه الصنف ايدن واعطى الصنف الشامي أعلى تراكيز معنوية للأحماض الدهنية ستيريك واللينوليك واللينوليك والأوليك وفيما يتعلق بالمحتويات الفينولية، تم اكتشاف أحماض الكافيين، الإيلاجيك، و p-hydroxybenzoic بتركيزات أعلى في الزعراني مقارنة بالصنفين الآخرين في حين كان تركيز حامض الجاليك اعطت حمض الجاليك فقط بتركيز أعلى في الصنف الشامي يليه صنف ايدن. ظهر استخدام المستوى الأعلى 100 P<sub>2</sub>O<sub>5</sub> كغم هكتار<sup>-1</sup> زيادة معنوية في إنتاجية الزيت مقارنة بالمستويات الأقل 0 و 50 P<sub>2</sub>O<sub>5</sub> كغم هكتار<sup>-1</sup>، علاوة على ذلك، فقد تجاوزت بشكل ملحوظ تركيبات الأحماض الدهنية واللينوليك واللينوليك والأوليك، ولكن المستويات الأعلى من P<sub>2</sub>O<sub>5</sub> زيد فقط من محتوى أحماض الكافيين والجاليك، بينما خفض محتوى حمض الإيلاجيك و p- هيدروكسي بينزويك. لم تكن استجابة تفاعلات الأصناف لتطبيقات P<sub>2</sub>O<sub>5</sub> معنوية لمعظم صفات الدراسة باستثناء تركيبة الحامض الدهني، ومحتوى أحماض الإيلاجيك، والجاليك، و p- هيدروكسي بينزويك..

الكلمات المفتاحية: اصناف العنبر، اضافة الفسفور، حاصل الزيت، الاحماض الدهنية، حامض الفينول.

## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the most important annual edible oilseed crops grown at winter season in the world (1,2) belonging to the family Asteraceae (Compositae), and most suitable for dryland farming due to its important characteristics like drought resistance because of their deep root system and short duration, also they are cultivable in all types of soil with low inputs and grows on residual soil moisture (3). After soybean, groundnut, rapeseed, sunflower, sesame, linseed and castor safflower ranks eighth regarding the total harvested yield. According to the 2014 FAO statistics, safflower production in the world was realized on an area more than one million hectares with a total world production reaching more than 867 thousand tons. Its oil is widely utilized in many industries for edible and dying purposes (4). The seeds of safflower are rich in oil (20 – 40%) with higher levels of unsaturated fatty acid (oleic and linoleic acid) about 90% (5,6,7,8) and protein (15 – 20%) and therefore are of great nutritive value for human and animal consumption. In Safflower, some physiological

traits like growth, seed quality and oil content are controlled by genotype, ecology, morphology, physiology and chemical fertilizer application (9,10). The Environmental condition influenced severely on oil yield, and oil content, the oil yield ranged between 226-1313 kg ha<sup>-1</sup> in winter cultivation, while in summer cultivation ranged between 1412-2140 Kg ha<sup>-1</sup> as average of some varieties of safflower (11). phosphorus (P) play a vital role in crop yield (12), it increases oil concentration in oil seed crops (13). With regard to the effects of phosphorus (P) fertilization on canola, reports showed that phosphorus is required with large quantities, especially in meristemic tissues, where cells are rapidly dividing and enlarging (14). The phosphorus fertilization helps in energy storage, early maturity of crops and root development (15). The functional and nutritional values of different vegetable oils are dependent on the nature of the different fatty acids, which are incorporated into the oil triacylglycerol's (16), Seeds of safflower contain higher unsaturated fatty acids than other oil seed plants. The oil contains two main unsaturated fatty acids: 13% oleic acid (18:1n9) (omega 9), 77% Linolic

acid(18:2n6) (omega6) which are present 90% of the total fatty acid content, and the linolenic acid (18:3n3) (omega 3) is exist in trace amount, while the remaining 10% corresponds to saturated fatty acids, such as palmitic and stearic acids (17,18). In recent years, safflower has been widely investigated by the fields of genetic breeding and engineering through the manipulation and combination of the major genes that control fatty acid levels in the oil (19, 20). On the base of the oleic and linoleic contents, two types of safflower can be distinguished, which are a high oleic acid type, with an oleic acid content of up to 90.60% and a high linoleic acid type, with a linoleic acid content of up to 88.8% (21). Traditional safflower oil, rich in polyunsaturated linoleic acid is valued for human health reasons as the high linoleic content leads to significant reductions in blood cholesterol levels (22,17) although not suitable for prolonged frying due to low oxidative stability at high temperatures. On the contrary, oil which is rich in monounsaturated oleic acid shows high oxidative stability, which makes it suitable for food purposes and an alternative to olive oil in arid and semi-arid regions in the world (23,24,25). Moreover, oleic acid is characterized by a high single point of unsaturation and this represents an attractive characteristic to industry due to the large range of potential chemical products.

Oleic-rich safflower oil can be used in the production of varnishes, alcohols, paints, lubricants, cosmetics, detergents and bio-based plastics (17). It is clear from literature that the genotype factor significantly affects oil yield fatty acid synthesis and proportions of oleic and linoleic acids during oil formation in the seed. However, at the same time, the ratio of oleic to linoleic acid in the seed oil is also dependent upon environmental variables, such as moisture and temperature, furthermore the availability of important nutrient especially phosphorus during seed maturation (20). As a consequence, genotype–environment interactions greatly

influence the composition of safflower oil, as confirmed by various authors ([27,28,29). Edible oil extracted from a wide range of plants content a number of phenolic compounds, such as a caffeic acid, Ellagic acid, Gallic acid and P-Hydroxybenzoic acid, while are a class of micronutrient, contribute to oxidative stability, they have many health benefits, including anti-inflammatory, anti-cancer and antiviral abilities (30). The aim of the research was showing the range of variation in the oil yield and fatty acids content depending upon application of phosphorus fertilization of three safflower cultivars and their interactions, under the rain fed condition.

## MATERIALS AND METHODS

### Field site description:

This study carried out in Sulaimani Governorate / Kurdistan Region, at the Experimental Farm of the College of Agricultural Sciences, University of Sulaimani at Bakrajo, which located in the southwest of Sulaimani city (Latitude: 35° 33' N; Longitude 45° 27' Est altitude of approximately 830 m) during the winter season of 2017-2018. The experiment was containing 2 factors, first: Three safflower varieties were selected for cultivation, which has been provided by the Sulaimani Agricultural Research Center, namely; (Zaafarani, Aidn and; Al-shamia) and the second factor was different levels of Phosphorus fertilizer (0, 50 and 100 Kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) from (Triple Supar Phosphate) TSP 46%. the experiment conduct in Completely Randomized Block Design(CRBD) with split block arrangement each treatment combination repeated three times.

Each main plot was consisted of three subplots with four rows (0.40 m between rows and 0.20 m between plants); thus, the plant population was 142,857plant/ha. Planting date was on 12th December of 2016. The cultural operations and weed control were accomplished according to normal field practices. Half of recommended

dose of fertilization were added to the whole experiment which were 50 Kg Nitrogen/ha as urea and divided into two equal doses and were applied at the seeding time and after 20 days from germination.

### Seed oil determination:

Two grams of the harvested seed of each treatment was powdered by electric blender. Digital Soxhlet instrument used for oil distillation, with n-hexane solvent (BDH, UK), (31) the oil content calculated as follows:

$$\text{Oil percent} = [(W2-W1) \times 100] / S$$

W1 = weight of empty flask (g).

W2 = weight of flask and extracted oil (g)

S = weight of sample.

### Oil yield(Kg/ha):

The oil yield( Kgha<sup>-1</sup>) is the product of seed yield (Kgha<sup>-1</sup>) in oil divided by one hundred according by (32) as shown in the following equation: -

$$\text{Oil yield (kg ha-1)} = \frac{\text{Oil content (\%)}}{100} \times \text{Seed yield (kg ha-1)}$$

### Separation of fatty acids:

Separation of fatty acids was done using High Performance Liquid Chromatography HPLC (College of Agriculture / Salahaddin University-Erbil), on reversed phase C-8 (50×2.6mm ID) column. 3µm particle size, mobile phase was acetonitril: tetrahydrofuran: 0.1 percent phosphoric acid (51:37:12v/v), the flow rate 1mlminute<sup>-1</sup>. The eluted peaks were mentioned by UV detector set at 215 nm, and quantitative analyzed by comparing the area of well-known standard with the area of the sample under the same separation condition (33).

$$\text{Conc. of sample } \mu\text{gml}^{-1} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conce. Of standard} \times \text{dilution factor}$$

### Statistical Analysis:

The data were statistically analyzed according to the methods of analysis of variance as a general test; all possible comparisons among the means were carried out by using Least Significant Difference (L.S.D) test at significant levels of 0.05 and 0.01(34).

## RESULTS AND DISCUSSION

Data Obtained results in Table1 shows the effect of the safflowers variety on oil yield, fatty acid, and some phenolic acid content, regarding the oil yield Kgha<sup>-1</sup>, although statistically the differences between them were not significant, but the highest oil yield was 540.484 Kgha<sup>-1</sup> gained by Al-Shamia exceeded by approximately 50 Kgha<sup>-1</sup> when compared to the lowest oil yield gained by Aidn variety. However, the average of oil yield by the three varieties was 513.213 Kgha<sup>-1</sup> and this yield is in accordance with the finding ranges of oil yield in winter cultivation by (11).

While, the estimated fatty acid contents such as palmitic and stearic acid in safflower oil according to (17,18) was 10%, the data in Table 1 refers that palmitic not detected, and stearic acid was very low, which reached to 1.17% as average of the three varieties, and the varieties significantly differ between themselves significantly differ in stearic acid concentration, as the Al-Shamia recorded 1.238% the highest significant concentration of stearic acid, the lowest level 1.109% was detected in Zaaferani variety and the Aidn variety was in between.

Regarding the monounsaturated fatty acid oleic, and the polyunsaturated linoleic and linolenic acids, the obtained results in Table 1 represents high significant differences between the varieties. The linolenic, linoleic, and oleic acids

0.212%, 46.164%, and 19.667% respectively recorded in Al-Shamia were predominated significantly compared to other varieties. Calculating the averages of fatty acids of the three varieties seems to be similar to those records by references (17,18), linolenic acid average was 0.189% it is consider as a trace, linoleic acid and oleic acid 44.034% and 17.518% respectively represented approximately the ratio 3:1 a good quality indicator.

Similar to many edible oils extracted from plants the data in Table 1 shows trace concentrations of some phenolic acids, they were statistically differ between the three varieties, such as caffeic acid, ellagic acid, and p- hydrobenzoic with high significant

concentrations detected in Zaafarani variety which were 0.143%, 0.268%, and 0.729% respectively compared to lowest concentrations of caffeic and ellagic acids 0.005%, and 0.180% respectively in Al-Shamia and the lowest concentration 0.234% of p- hydroxybenzoic acid that detected in Aidn variety, another phenolic acid detected in safflowers variety was gallic acid that records the highest concentration 0.182% in Al-Shamia variety followed by 0.179% detected in Aidn and the lowest concentration of gallic acid was 0.268% detected in Zaafarani variety. Hence the safflower oil considers possessing medical properties (30).

**Table 1: Effect of safflower varieties on oil yield, fatty acid, and phenolic acids contents.**

<i>Varieties</i>	<i>oil yield kg ha<sup>-1</sup></i>	<i>Stearic acid %</i>	<i>Linolenic acid omega3 %</i>	<i>Linoleic acid omega6 %</i>	<i>Oleic acid omega9 %</i>	<i>caffeic acid %</i>	<i>Ellagic acid %</i>	<i>Gallic acid %</i>	<i>p-hydroxybenzoic acid</i>
<i>Zaafarani</i>	507.685	1.109	0.173	43.833	18.667	0.143	0.268	0.097	0.729
<i>Aidn</i>	491.470	1.171	0.184	42.106	14.222	0.007	0.198	0.179	0.234
<i>Al-shamia</i>	540.484	1.238	0.212	46.164	19.667	0.005	0.180	0.182	0.728
<i>LSD 0.05</i>	<b>n.s</b>	<b>0.011</b>	<b>0.015</b>	<b>0.436</b>	<b>1.051</b>	<b>0.025</b>	<b>0.008</b>	<b>0.012</b>	<b>0.046</b>
<i>LSD 0.01</i>	<b>n.s</b>	<b>0.016</b>	<b>0.020</b>	<b>0.601</b>	<b>1.448</b>	<b>0.035</b>	<b>0.011</b>	<b>0.016</b>	<b>0.064</b>

The results in Table 2 indicate that application of fertilizer has a highly significant effect on the oil yield and all fatty acids composition with the exception of caffeic acid. The treatment of 100 Kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> dominated among other levels application for increasing the amount of oil yield and all fatty acids with value of 620.942 kg ha<sup>-1</sup>, while the treatment of control indicates the minimum amount of oil

yield with the value of 418.940 kg ha<sup>-1</sup>, this result is confirm the role of phosphorus (P) in crop yield, it increase oil concentration in oil seed crops (12,13). The application of 100 Kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> dominated significantly all fatty acid and phenolic composition except Ellagic acid and p-hydroxibezanic acid, with the values 1.221 %, 0.213%, 45.416 %, 20.222%, and 0.176 %, for the fatty acid composition stearic acid ,

omega3, omega6, omega 9 and Gallic acid respectively, while the control treatment recorded the maximum value for the composition of Ellagic acid and p-hydroxybenzoic acid with 0.267 % and 0.169 % respectively, but gave the lowest values for the

other fatty acid composition with 1.123 %, 0.168%, 42.481%, 14.556% and 0.131% for stearic acid , omega3 ,omega6, omega 9 and Gallic acid respectively.

Table 2: Effect of phosphorus application on safflower oil yield, fatty acid, and phenoli.

<i>Phosphorus fertilizers levels (Kgha<sup>-1</sup>)</i>	<i>oil yield kgha<sup>-1</sup></i>	<i>stearic acid %</i>	<i>Linolenic acid omega3 %</i>	<i>Linoleic acid omega6 %</i>	<i>Oleic acid omega 9 %</i>	<i>caffeic acid %</i>	<i>Ellagic acid %</i>	<i>Gallic acid %</i>	<i>p-hydroxybenzoic acid</i>
<b>0</b>	418.940	1.123	0.168	42.841	14.556	0.037	0.267	0.131	0.691
<b>50</b>	499.756	1.173	0.187	43.847	17.778	0.056	0.210	0.151	0.577
<b>100</b>	620.942	1.221	0.213	45.416	20.222	0.062	0.169	0.176	0.423
<b>LSD 0.05</b>	<b>61.199</b>	<b>0.011</b>	<b>0.015</b>	<b>0.436</b>	<b>1.051</b>	<b>0.000</b>	<b>0.008</b>	<b>0.012</b>	<b>0.046</b>
<b>LSD 0.01</b>	<b>84.320</b>	<b>0.016</b>	<b>0.020</b>	<b>0.601</b>	<b>1.448</b>	<b>n.s</b>	<b>0.011</b>	<b>0.016</b>	<b>0.064</b>

Table 3 showed non-significant variation of oil yield and some fatty acid composition between the treatment combinations. But there was highly significant variation of some fatty acid composition analysis due to interaction treatment between fertilization application and varieties. Concerning stearic acid, the maximum value with 1.300 % was recorded by interaction between Al-Shamia variety and 100 Kgha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and the minimum value with 1.073 % was recorded by the interaction between Zaafarani variety and control treatment. Regarding Ellagic acid recorded maximum value with 0.337% due to interaction between Zaafarani variety and the

treatment of control, and the minimum value with 0.167% were recorded by the interaction between Al-Shamia variety and 100 Kgha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. Gallic acid produced maximum value with 0.210 % due to interaction of Aidn × 100 Kgha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and the minimum value with 0.077 % was recorded by Zaafarani × zero P<sub>2</sub>O<sub>5</sub>. The interaction between Zaafarani × zero P<sub>2</sub>O<sub>5</sub> gave maximum value with 0.900 % p-hydrobenzoic acid, while the lowest value with 0.043 % were recorded by the combination of Aidn × 100 Kgha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>.

Table 3: Effect of interaction between fertilization and safflower varieties on safflower oil yield, fatty acid, and phenolic acids content.

<i>Varieties × P2O5 Kgha-1</i>	<i>oil yield kgha<sup>-1</sup></i>	<i>stearic acid %</i>	<i>Linolenic acid omega3 %</i>	<i>Linoleic acid omega6 %</i>	<i>Oleic acid omega9 %</i>	<i>caffeic acid %</i>	<i>Ellagic acid %</i>	<i>Galllic acid %</i>	<i>p- hydroxybenzoic acid</i>
<i>Zaafarani × 0</i>	405.39 5	1.073	0.152	42.883	15.33 3	0.102	0.337	0.077	0.900
<i>Zaafarani × 50</i>	467.88 2	1.090	0.174	43.500	18.66 7	0.157	0.280	0.087	0.737
<i>Zaafarani × 100</i>	649.77 8	1.163	0.191	45.117	22.00 0	0.170	0.187	0.127	0.550
<i>Aidn × 0</i>	431.90 3	1.123	0.169	40.887	10.66 7	0.004	0.270	0.143	0.390
<i>Aidn × 50</i>	475.22 9	1.190	0.186	41.927	15.00 0	0.006	0.170	0.183	0.270
<i>Aidn × 100</i>	567.27 8	1.200	0.196	43.503	17.00 0	0.009	0.153	0.210	0.043
<i>Al-shamia × 0</i>	419.52 4	1.173	0.183	44.753	17.66 7	0.004	0.193	0.173	0.783
<i>Al-shamia × 50</i>	556.15 8	1.240	0.201	46.113	19.66 7	0.005	0.180	0.183	0.723
<i>Al-shamia × 100</i>	645.77 1	1.300	0.252	47.627	21.66 7	0.006	0.167	0.190	0.677
<i>L.S.D (P ≤ 0.05)</i>	<b>n.s</b>	<b>0.020</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>0.014</b>	<b>0.020</b>	<b>0.080</b>
<i>L.S.D (P ≤ 0.01)</i>	<b>n.s</b>	<b>0.027</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>n.s</b>	<b>0.020</b>	<b>n.s</b>	<b>0.110</b>

## CONCLUSION:

Obtained results of this study showed that in spite of the differences between the safflower varieties approximately 500 Kgha<sup>-1</sup> was the average of oil yield of the three varieties, it is acceptable for cultivation under the rainfed condition of Sulaimani/Kurdistan region. Regarding to the quality of the oil of these varieties and on the base of linoleic to oleic ratio which was close to 3:1 consider to be a good

quality. In addition to fatty acid contents, the oil of the studied varieties were contained in trace concentrations of some phenolic acid which have benefits to human health, this provides medical properties to the safflower oil of these varieties. The results also confirmed the great influence of phosphorus nutrient in improving the growth, yield, and yield components of the safflower, ultimately increasing oil quantity and quality.

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