

DOI: <http://dx.doi.org/10.21123/bsj.2022.19.2.0240>

## Effect of different levels and sizes of walnut seed residues on phosphorus availability and alkaline phosphatase activity in calcareous soil

Sakar Abdulqadr Saheed<sup>1\*</sup>      Shakar Jamal aweez<sup>1</sup>      Snowber Muhamad Ahmed<sup>2</sup>

<sup>1</sup>Department of environmental science, College of Science, University of Salahadden, Iraq.

<sup>2</sup>Department of general science, college of basic education, University of Sallahaddin, Iraq.

\*Corresponding author: [sakar-saheed@su.edu.krd](mailto:sakar-saheed@su.edu.krd), [shaker.aweez@su.edu.krd](mailto:shaker.aweez@su.edu.krd), [snowber.ahmed@su.edu.krd](mailto:snowber.ahmed@su.edu.krd).

\*ORCID ID: <https://orcid.org/0000-0002-7820-6223>, <https://orcid.org/0000-0003-0469-1315>, <https://orcid.org/0000-0001-8744-7419>.

Received 28/1/2020, Accepted 10/11/2020, Published Online First 20/9/2021, Published 1/4/2022



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

### Abstract:

A laboratory experiment has been carried out in the College of Science-University of Salahaddin to study the effect of different levels (0,5,10 and 15%) and sizes(250 and 1000 $\mu$ m) of walnut seeds residues and (160mg.kg<sup>-1</sup>) phosphorus fertilization on the concentration of phosphorus availability and alkaline phosphatase activity in calcareous soil during 15 and 30 days period of incubation, the experimental design in factorial complet randomize design (C.R.D) with three replications. The results indicated that the application of different levels of walnut seed residues decreases the concentration of phosphorus availability and alkaline phosphatase activity, however the results revealed that combination between levels and sizes of sieved walnut seed residues and phosphorus fertilizer significantly ( $p \leq 0.05$ ) affected the alkaline phosphatase activity and the concentration of available phosphorus, the highest values (29.633  $\mu$ gPNPg<sup>-1</sup>.hr<sup>-1</sup> and 6.442mg.kg<sup>-1</sup>) have been recorded in treatments combination received 1000 $\mu$ m, 15% walnut seed residues and phosphorus fertilizer, respectively.

**Keywords:** Alkaline phosphatase, Available phosphorus, Fertilization, Walnut seed residues

### Introduction:

The soil is a living dynamic system containing many free enzymes, immobilized extra-cellular enzymes within enzymes inside the microbial cells. The soil enzymes include a wide spectrum of oxidoreductases, transferase, hydrolyzes and lyses. Enzymes present in soil are like enzymes in other systems<sup>1</sup>. Enzymes are viewed to be an important soil components, their activities in soil have the potential to provide unique biological information of soils; along these lines, they are alluring as one proportion of soil health<sup>2</sup>. Phosphatase is an enzyme of great agronomic value because it hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus, which is assimilating by plants<sup>3</sup>. Plant residues application is an important way to maintain soil productivity. The walnut (*Juglans regia* L.) is the most trees widespread around the world, particularly in the Kurdistan region. Walnut is nutrient-dunce food mainly owing to the fact that it is fat contetnt as

well as protein, vitamin and mineral profiles, also estimated that 70% of the fruit weight is husk and shell<sup>4</sup>.

Phosphorus is an essential element for plant growth and other living organisms such as microbes and havea role in energy transport, cellular structures, and nucleic acids and is thus essential for life<sup>5,6</sup>. while calcareous soil is widespread through the word involved Kurdistan region soils, thus the availability of phosphorus in these soils are low due to the high calcium carbonate (CaCO<sub>3</sub>) which lead to chemical fixation of phosphorus as well as the large quality of walnut seed residues particularly the hard parts of seed discharge to the landfill as solid waste without taking benefiting of it in economic and agricultures term. Thus, the aim of this research is to determine the availability of phosphorus and alkaline phosphatase activity of calcareous soil after treating the soil by walnut residue.

## Materials and Methods:

A laboratory experiment has been carried out in the College of Science-University of Salahaddin to study the effect of different levels and sizes of walnut seeds residues and phosphorus fertilization on the concentration of phosphorous availability and alkaline phosphatase activity in calcareous soil, the experimental design in factorial CRD (Complete Randomise Desigen) with three replications. The soil used in this research was taken in Debaga location, some characteristics of studied soil are presented in Table (1). The first factor involves walnut residues sieved in 250 $\mu$ m and 1000 $\mu$ m, the second factor is walnut residues dose (0, 5, 10 and 15%), the third factor involves the application of triple superphosphate (160mg/kg) and without fertilizer application. Hence 16 experimental combination treatments have been tested in every three replicates making a total of 48 combination treatments that have been incubated at 25°C for 15 and 30 days, after incubation the treatments analyses for.

**Table 1. Some physical and chemical properties of soil used in the study .**

Particle Size Distribution (PSD) g Kg <sup>-1</sup>	Sand	270
	Silt	416
	Clay	314
Textural name	Clay loam	
pH	7.7	
EC (dS m <sup>-1</sup> ) at 25°C	0.63	
Organic Matter g Kg <sup>-1</sup> soil	1.1	
Total CaCO <sub>3</sub> g Kg <sup>-1</sup> soil	350	
Total Nitrogen % soil	0.35	
Total Phosphorus mg .Kg <sup>-1</sup> soil	260	
available Phosphorus ppm soil	1.1	
Soluble ions mmolc L <sup>-1</sup>	Ca <sup>2+</sup>	1.6
	Mg <sup>2+</sup>	0.42
	Na <sup>1+</sup>	1.01
	K <sup>1+</sup>	1.68

## Available phosphorus:-

The available phosphorus was determined by Olsen-extractable and stannous chloride methods

using the spectrophotometer at (730nm) as described by <sup>7</sup>.

## Alkaline phosphatase

The Alkaline phosphatase has been determined using p-nitrophenyl phosphate substrate made in a buffer solution with pH=11, 1g of moist soil has been treated with 0.25ml of toluene, 4 ml modified buffer (pH=11), 1 ml of p-nitrophenyl phosphate made in the same buffer, mixed and incubated for 1h at 37°C. After the incubation, 1ml of 0.5M CaCl<sub>2</sub> and 4ml of 0.5M NaOH have been added, and the content has been mixed and filtered through a filter paper. The absorbance in the filtrate has been measured at 400 nm using a spectrophotometer as described by <sup>8</sup>

## Results and Discussion

Data present in Fig. (1) indicates that walnut residual levels significantly ( $p \leq 0.05$ ) diminished the concentration of available phosphorus and action of phosphatase enzymes in calcareous soils. The highest values (5.261mg.kg<sup>-1</sup> and 25.073 $\mu$ gPNP<sup>-1</sup>.hr<sup>-1</sup>) have been recorded in control (W0%), while the lowest values (3.064 mg.kg<sup>-1</sup> and 22.388 $\mu$ gPNP<sup>-1</sup>.hr<sup>-1</sup>) have been recorded in case of application the (W<sub>1</sub> 5% ) respectively. Beyond this level of walnut residues, the concentration of available phosphorus and the action of alkaline phosphatase gradually has increased with increment the levels of walnut residues. This result indicates that organic residues may play central roles enhancing the adsorption capacity of soil to phosphorus also the decomposition of the amended residues releases basic cations like calcium , magnesium , or trace elements such as the Fe that react with phosphorus which lead to form insoluble compounds. This result and deciphering partially agreed with those reported by <sup>9</sup> shows that crop residues can improve soil quality by increasing soil organic matter advanced soil structure formation, expanding soil microbial biomass, and enhancing various enzymatic activities. The utilize of organic materials, including plant residues, to amend rock phosphate has been found to extend the availability of P from the phosphorous rock in soils, that application of plant residues increments the availability of native soil P <sup>10</sup>.

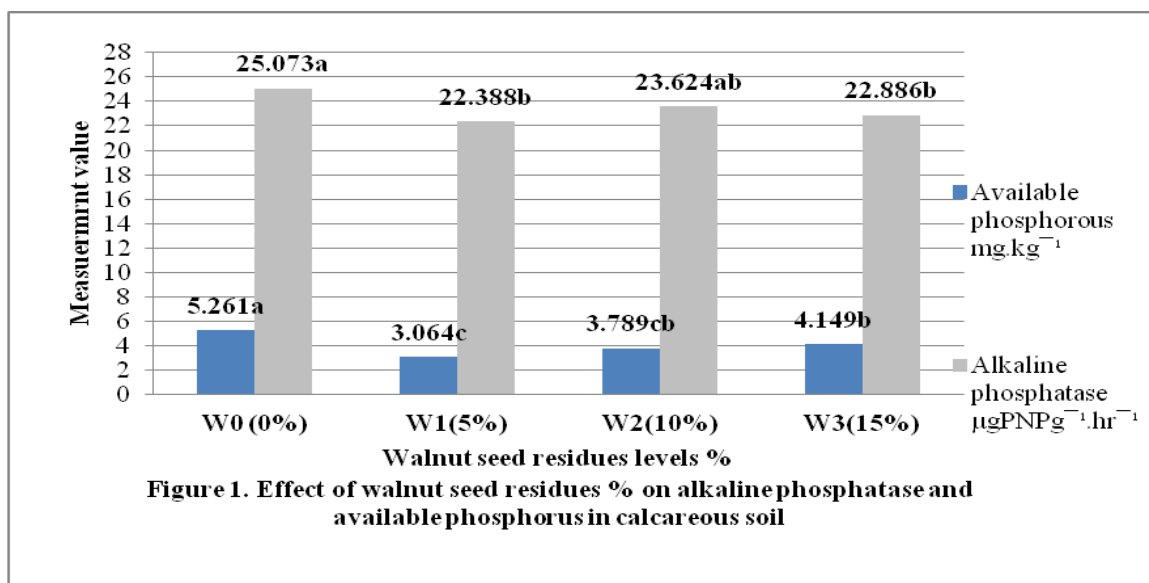


Figure 1. Effect of walnut seed residues % on alkaline phosphatase and available phosphorus in calcareous soil

Data present in Table (2) indicates that a combination between sieved walnut seed residues size, phosphorus fertilizer, and incubation periods significantly ( $p \leq 0.05$ ) affected the alkaline phosphatase and available phosphorus in calcareous soils. The highest values (29.52 and 5.6mg.kg<sup>-1</sup>) have been recorded in combination treatments (S<sub>2</sub> P<sub>2</sub>D<sub>1</sub>) and (S<sub>1</sub>P<sub>1</sub>D<sub>2</sub>), while the lowest values (18.394µgPNP<sup>-1</sup>.hr<sup>-1</sup> and 3.275mg.kg<sup>-1</sup>) have been recorded in case of combination treatments (S<sub>2</sub> P<sub>1</sub>D<sub>2</sub> and S<sub>1</sub> P<sub>1</sub>D<sub>1</sub>), respectively. This result indicates that applying phosphorus fertilizer with walnut residues for short time enhanced phosphatase enzymatic activity, while the application of fine particles walnut seeds residues increase the concentration of phosphorus availability. The fertilization is a significant rural practice for improving nourishment of plants, reaching high yield, and furthermore, for changes of soil environment, such as, the chemistry of soil carbon and nitrogen, which can change the activity and diversity of soil microorganisms. Microorganisms may react to changing states of soil environment by modification of composition in microbial community and the total quantity of

microbial biomass. Additionally, changes in microbial community influence transformation of C and N in the soil ecosystems by along these lines enhanced enzymatic activity. Likewise availability phosphorus increment by application fertilizer may be due to the fact that soils pH values brought by walnut application down to values are perfect for P-availability, while the higher levels of walnut seed residues might be raising the pH values that restrict the P-availability besides the organic fertilizer upkeep is an important to factor in controlling phosphorus availability. This result is similar to those<sup>11</sup> that indicate combined fertilization that may improve biological characteristics in deeper parts of the soil profile and possibly increase biological activity in agroecosystems, alkaline phosphates have been increased to various degrees by all liming materials, especially on the silty clay. The increment in P concentration in the soil solution by fertilizer expansion upgrade the phosphate ions absorbed by roots, microbial biomass, or retained through sorption-desorption precipitation dissolution, immobilization and mineralization mechanisms<sup>12</sup>.

Table2. Combination effect of sieved walnut seed residues size, phosphorus fertilizer and incubation periods on alkaline phosphatase and available phosphorus in calcareous soils:

Sieved walnut seed residues (µm)	Phosphorous fertilizer	Incubation days	Alkaline phosphatase µgPNP.g <sup>-1</sup> .hr <sup>-1</sup>	Available phosphorous mg.kg <sup>-1</sup>
S1(250µm)	P1(0 mg.kg <sup>-1</sup> )	15	24.338 <sup>b</sup>	3.275 <sup>b</sup>
		30	18.519 <sup>d</sup>	5.6 <sup>a</sup>
	P2(160 mg.kg <sup>-1</sup> )	15	24.804 <sup>a</sup>	3.896 <sup>b</sup>
		30	20.881 <sup>cd</sup>	3.822 <sup>b</sup>
S2(1000µm)	P1(0 mg.kg <sup>-1</sup> )	15	23.623 <sup>bc</sup>	3.326 <sup>b</sup>
		30	18.394 <sup>d</sup>	4.833 <sup>ab</sup>
	P2(160 mg.kg <sup>-1</sup> )	15	29.52 <sup>a</sup>	4.21 <sup>ab</sup>
		30	22.125 <sup>bc</sup>	4.714 <sup>ab</sup>

The same litter referred to non-significant effect, while the different litters referred to significant effect.

Data analysis in Table (3) indicates that a combination between levels and sizes of sieved walnut seed residues and phosphorus fertilizer significantly ( $p \leq 0.05$ ) affected the alkaline phosphatase activity and the concentration of available phosphorus in calcareous soils. The highest values ( $29.633 \mu\text{gPNP}^{-1} \cdot \text{hr}^{-1}$  and  $6.442 \text{mg} \cdot \text{kg}^{-1}$ ) have been recorded in treatment combination ( $S_2W_0P_1$  and  $S_2W_3P_2$ ), respectively while the lowest values of ( $15.001 \mu\text{gPNP}^{-1} \cdot \text{hr}^{-1}$  and  $1.376 \text{mg} \cdot \text{kg}^{-1}$ ) have been recorded in case of treatments combination ( $S_2W_3P_1$  and  $S_2W_2P_1$ ) treatment, respectively. These results show that plant residue provides an increase in surface area of soil particles at that point which improve the association phosphatase to clay fraction in soil and

decline the enzyme activity that has been identified with the way that the activity of clay adsorbed enzyme is lower than free enzyme; however, the utilization of walnut residues to soil encouraging microbial colonization because the plant residues manage soil moisture, temperature, aeration, pH, the carbon-nitrogen ratios in which have an important role for microbial activity, also enhanced availability nutrient also the P deficiency enhanced the activity of the phosphate from fungi and other microorganisms. These results agree with those that have been reported by <sup>13</sup>. They reported that the addition of organic residues can stimulate enzymatic activity as a result of microbial proliferation or enzymatic induction in response to the added residues. Available nitrogen, phosphorus, and potassium in soil was significantly ( $p=0.05$ ) increased with different rates of application of plant residues level<sup>14</sup>.

**Table 3. Combination effect of sieved walnut seed residues size, Walnut residues levels and phosphorus fertilizer on alkaline phosphatase and available phosphorus in calcareous soil.**

Sieved walnut seed residues ( $\mu\text{m}$ )	% Walnut seed residues	Phosphorous fertilizer	Alkaline phosphatase $\mu\text{gPNPg}^{-1} \cdot \text{hr}^{-1}$	Available phosphorous $\text{mg} \cdot \text{kg}^{-1}$
S1(250 $\mu\text{m}$ )	W0 (0%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	23.459 <sup>bcd</sup>	5.803 <sup>abc</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	20.920 <sup>cd</sup>	4.259 <sup>abcd</sup>
	W2 (5%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	20.167 <sup>d</sup>	4.078 <sup>abcd</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	23.112 <sup>bcd</sup>	4.196 <sup>abcd</sup>
	W3 (10%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	23.019 <sup>bcd</sup>	4.476 <sup>abcd</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	27.541 <sup>ab</sup>	2.761 <sup>de</sup>
	W4 (15%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	19.069 <sup>de</sup>	3.393 <sup>cde</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	25.799 <sup>ab</sup>	4.222 <sup>abcd</sup>
S2(1000 $\mu\text{m}$ )	W0 (0%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	29.633 <sup>a</sup>	6.229 <sup>ab</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	26.28 <sup>ab</sup>	4.753 <sup>abcd</sup>
	W2 (5%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	20.634 <sup>cd</sup>	1.376 <sup>e</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	25.64 <sup>ab</sup>	2.603 <sup>de</sup>
	W3 (10%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	18.769 <sup>de</sup>	3.844 <sup>bcd</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	25.167 <sup>abc</sup>	4.05 <sup>abcd</sup>
	W4 (15%)	P1 (0 $\text{mg} \cdot \text{kg}^{-1}$ )	15.001 <sup>e</sup>	4.87 <sup>abcd</sup>
		P2 (160 $\text{mg} \cdot \text{kg}^{-1}$ )	26.207 <sup>ab</sup>	6.442 <sup>a</sup>

The same litter referred to non-significant effect, while the different litters referred to significant effect

### Conclusions:

The application of 5% levels of walnut seed residues significantly decreased the concentration of available phosphorous and the activity alkaline phosphatase respectively. Beyond this level of walnut residues, the concentration of available phosphorus and the action of alkaline phosphatase gradually increased with increment the levels of walnut residues; however, the application of walnut seed residues with phosphorus fertilizer for short period which increases the concentration of available phosphorus and the action of alkaline phosphatase in calcareous soil.

### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Salahadden.

### Authors' contributions statement:

All three authors play important roles to complete different part of this research in good form. Sakar and Shakar authors are performed all

practical parts also wrote the paper and Snawber author statistically analysis the data and corrected the paper scientifically and grammatically. Finally, this research was carried out throw cooperation by researchers.

### Reference:

1. Stanislaw, k. and Barbara, S. Enzymatic Activity of soil after applying various waste organic materials, ash, and mineral fertilizers). *soil science and plant nutrition, Siedlice university of natural science and Humanities, prusa* 2012; 14, 08-110
2. Rankoth LM, Adawatta RP, Veum KS, Jose S, Alagele S. Cover crop influence in soil enzymes and selected chemical parameters for soybean rotation. *Agriculture*. 2019; 9, 125; doi: 10.3390/agriculture9060125.
3. Dindar E, Shagban FO, Baskaya HS. Evaluation of soil enzyme activity as soil quality indicator in sludge-amended soil, 2015. *J. Environ Biol*. 2015 Jul; 36(4), 919-26.
4. Jahanban-Esfahlan A, Ostadrahimi A, Tabibiazar M, Amarowicz R. A comparative Review on extraction, Antioxidant content and Antioxidant potential of different parts of walnut (*Juglans regia* L.) fruit and tree). *Molecules*. 2019; 24:2133.
5. Jakubauskaite I, Karcauskiene D, Antanaities S, Mazvila J, Slepetic A, Koncius D, et al. The distribution of phosphorus forms and fraction in Retisol under different soil liming management. *Zemdirbyste-Agriculture*, 2015; 102(3), p.215-256; doi 10.13080/z-a.
6. Al-Rohily KM, Ghoneim AM, Modaihsh AS, Mahjoub MO. Phosphorus availability in calcareous soil amend with chemical phosphorus fertilizer, cattle manure compost and sludge manure). *Int. J. Soil Sci.*, 2013; 8(1): p.17-24; doi:10.3923/ijss.2013.17.24.
7. Allen SE. Chemical analysis of ecological materials. Oxford: Blackwell scientific publication Osney Mead; 1974. p.64-214.
8. Alef K., Nannipieri P., Trazar-Cepeda C. Phosphatase activity. In: Alef, K., Nannipieri, (Eds), *Method in Applied Soil Microbiology and Biochemistry*. London: Academic press; 1995, p.335-344.
9. Zhao B, Zhang J, Yu Y, Karlen DL, Hao X. Crop residue management and fertilization effects on soil organic matter and associated biological properties. *Environ Sci Pollu Res*. 2016; 23:p.17581–17591.
10. Kolawole GO, Adigun CO, Akintade BO, Opaleye, OA, Lawal BA. Organic Residues Affect Soil P Availability, Cowpea Yield and Nutrient Uptake on a Near Neutral P-Deficient Alfisol in Southwestern Nigeria. *IJAAR*. 2013;9 (1&2): p.81-90.
11. Holík L, Hlisenikovsky L, Honzík R, Trögl J, Burdová H, Popelka J. Soil Microbial Communities and Enzyme Activities after Long-Term Application of Inorganic and Organic Fertilizers at Different Depths of the Soil Profile. *Sustainability*; 2019; 2019 Jan; 11(12):3251
12. Zamuer EC, Picone LI, Diez AB. Effect of long-term phosphorus fertilization on soil phosphorus fractions. *SJSS* 2012; 2(2):50-61. 12. 12.
13. Bautista-Cruz A, Ortiz-Hernández DY. Hydrolytic soil enzymes and their response to fertilization: a short review. *Comunicata Scientiae*. 2015; Jun 6(3). P.255-262
14. Ravi S, Jadhav RL, Sidramappa R, Kamble A. Effect of Plant Residues on Chemical Properties of Soil and Nutrient Uptake of Soybean. *Int J Curr Microbiol Appl Sci*. 2017 Dec 6(12): p.3484-3489

## تأثير المستويات والاحجام المختلفة من بقايا بذور الجوز على الفوسفور الميسر ونشاط الفوسفاتيز القلوي في التربة الكلسية

صنوبر محمد احمد<sup>2</sup>

شاكار جمال عويز<sup>1</sup>

ساكار عبدالقادر سعيد<sup>1</sup>

<sup>1</sup>قسم علوم بيئة ، كلية علوم ، جامعة صلاح الدين، اربيل، عراق  
<sup>2</sup>قسم علوم العامة، كلية تربية الاساس، جامعة صلاح الدين، اربيل، عراق

### الخلاصة:

تمت إجراء تجربة مختبرية في كلية العلوم-جامعة صلاح الدين لدراسة تأثير المستويات المختلفة (0 ، 5 ، 10 و 15 %) والاحجام (250 و 1000  $\mu\text{m}$ ) من بقايا بذور الجوز و (160 mg.kg) من سماد الفوسفاتي على تركيز الفوسفور الميسر ونشاط الفوسفاتيز القلوي في التربة الكلسية خلال فترة (15 إلى 30) يوماً من الحضنة ، والتجربة المصممة في CRD بثلاثة مكررات. وأشارت النتائج إلى ان تطبيق المستويات المختلفة من بقايا بذور الجوز يسبب من تركيز الفوسفور الميسر والنشاط الفوسفاتيز القلوية. ومع ذلك ، كشفت النتائج ان الجمع بين المستويات والاحجام من بقايا بذور الجوز والسماد الفوسفاتي بشكل ملحوظ ( $p \geq 0.05$ ) أثرت على النشاط الفوسفاتيز القلوي وتركيز الفوسفور الميسر ، وأعلى القيم (29.633  $\mu\text{gPNPg}^{-1}.\text{hr}^{-1}$  و 6.442 mg.kg)، سجلت في المعاملة العاملة 1000  $\mu\text{m}$  و 15% من بقايا بذور الجوز مع سماد فوسفاتي على توالي.

**الكلمات المفتاحية:** الفوسفاتيز القلوي ، الفوسفور الميسر ، السماد الفوسفاتي ، بقايا بذور الجوز.