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Mamoon Shakir Zghair Altaee *
Rand Abdalhadi Ghazal Altaee

Department of Soil Sciences and Water Resources, College of Agriculture and Forestry, University of Mosul

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Influence of substrate concentration on kinetic parameters of alkaline phosphatase enzyme

ABSTRACT

A laboratory study was conducted in the laboratory of the Department of Soil Science and Water Resources in Agriculture and Forestry College and in the Faculty of Environment laboratory with the aim of showing the effect of temperature on the activity and effectiveness of the phosphatase enzyme and the calculation of kinetic and thermodynamic parameters (maximum velocity Vmax and concentration of the subject substance Km) and for the sake of So, it was soil chosen from Zawita forest, with sandy clay loam texture under the cover of pine trees. The soil was treated with different concentrations of Substrate subject to the enzyme (0.0125, 0.025, 0.05, 0.075, 0.1) molar and under the influence of different temperatures (10,20,30,40,50) C°.The results showed. A clear effect of the subject substance on the activity of the enzyme, as the enzyme effectiveness increased with the increase of the added concentrations of the substance. The highest efficacy value for alkaline enzyme phosphatase was V / [S] (0.6) µg P-nitrophenol gm-1. Hr-1, which resulted from the effect of concentration (100) mmol of the subject substance. While the lowest alkaline phosphatase enzyme effectiveness value V / [S] was (0.2) µg P-nitrophenol gm-1. Hr-1, which resulted from the effect of the lowest concentration of the subject substance (12.5) mmol. The results indicated thatV max value of amounted to (227.27) µg P-nitrophenol gm-1. Hr -1. while the value of Km amounted to (38.52) mmuler. The results showed an increase in enzyme phosphatase activity by increasing the incubation temperature at concentration (0.05) molar of the substrate, the highest value of the activity of the enzyme phosphatase reached V (155.47) mg P-nitrophenol gm -1 hr -1, which occurred from soil incubation at The highest temperature used in the study is (50) C°, as it was observed that the values of phosphatase enzyme effectiveness continued to increase until the highest temperature used in the study.

INTRODUCTION

Soil enzymes are the main key to the processes of analyzing organic compounds, nutrient readiness and other soil biochemical processes (Al-Jubouri, 2018). They are also auxiliary factors consisting of proteins of a precise specialty and possess stimulating properties that increase the rate of the reaction without changing the properties of the enzyme after the end of the reaction (Kizilkaya and Bayrakli 2005).

Enzymes are often used as indicators of soil fertility as it is very sensitive to environmental conditions and respond much more quickly to changes in soil management than other soil variables. It reflects the integrated biological assessment of soil function, especially those that stimulate a

* *Corresponding author: E-mail*: Randaltaee44@uomosul.edu.iq

wide range of processes such as Phosphatase, Urease, Dehydrogenase and Aspergenase (Gianfreda and Ruggiero, 2006).

The alkaline phosphatase enzymes are one of the hydrolytic enzymes that plays an important role in the phosphorous cycle, and it is essential for plant growth and development due to its direct role in most vital processes such as photosynthesis and respiration.The enzyme transforms organic phosphorus into available forms that contribute to plant growth through the phosphates hydrolysis associated with complex organic compounds or inorganic compounds then produces phosphoric acid and alcohol (Hui et al., 2013).

Using microbiology which is convert non-dissolved (unavailable) forms of some soil minerals into soluble (Available) forms has an important role in equivalent and alkaline soils. The previous studies have shown that the presence a high density of dissolved phosphate microbiology in the rizosphere zone with organic matter may not decompose or it will decompose slowly due to the lack of specialized organisms, or its presence is few due to the conditions that are not suitable for its reproduction, and it does not meet the mineralization of organic matter. Therefore, this specialized type will product in the laboratory and vaccinate the rizosphere zone to increase the number of organisms and increase their activity until they have the ability to Increasing the availability of some nutrients in neutral and alkaline soils. Microorganisms are often used in the form of bio-fertilizers to transform organic materials into mineral forms or to dissolve phosphates to be available for plants (Al-Shahat, 2007).

One of the most important benefits of vegetation cover for soil is to work on soil pedon development into different horizons and reduce the time for soil to reach the degree of maturity in addition to other soil formation factors such as climate, topography, parent material and time, where vegetation increases the amount of organic matter in the soil, which in turn It works to improve the different soil properties, due to the decomposition of the fallen leaves of the trees. The Pine trees are common forest trees compared to other species in order to their suitability to arid and semi-arid environmental conditions and their growth in rocky, mountainous and sandy lands, in addition to the It is importance of these trees in improving soil condition and reduce sand creep and environmental degradation. As well as provides suitable environments to protect wildlife and biodiversity, in addition to purifying the air inside cities, giving an aesthetic view of it Bait Al Mal (2010).

Due to that the forest soils contain organic residues with indications for soil fertility, Therefore, the study aims to

1.Studying the temperature effect on the phosphatase enzyme activity in soil.

2.Study the effect of the P-nitrophenol concentration on the phosphatase enzyme activity in the soil.

3.Estimation of the kinetic values of Vmax and Km for phosphatase enzyme in soil.

MATERIALS AND METHODS

Soil sampling and preparation:

Soil sample have been taken under Pine trees Zawita region at different dimensions from the tree trunk at (0-15) cm depth, The soil was transported to the laboratory with plastic bags, then air dried, grinded with a wood hammer, and finally sifted through a 2 mm diameter of holes and kept the soil in the refrigerator until using it in the experiments.

Soil physical and chemical analyses:

Physical analyses included:

- **Soil Texture:** Particle Size Distribution was estimated by hydrometer method with treating a known weight of soil with a Calcon, first value foe hydrometer are taken after 40 seconds and the second reading after two hours based on temperatures at each reading, according to Ryan and others,(2003).
- **Field capacity:** The moisture content was estimated using weighty method by saturate the soil with water then turning it into a funnel containing a filter sheet and left for 24 hours after that placed the sample in oven for 24 hours, then measure moisture content in the soil is measured, according to USDA, (2004)

Chemical analyses included:

- **Electrical conductivity (EC):** was determined by extract soil is taken soil: water (1:1) and measured by (EC meter) using (ST 740 Sony) according to USDA, (2004).
- **Soil Reaction (pH):** The negative logarithm of the hydrogen ion concentration (pH) (Soil reaction) was measured extract (1:1) soil:water using pH-meter (ST 740 Sony), as stated in (USDA, 2004).
- **Organic matter (O.M.):** The organic matter was estimated by organic carbon oxidation according to (Walkely and Black) using potassium dichromate (1 N), concentrated sulfuric acid and calibration with ammonium iron sulfate (0.5 N) to calculate excess dichromate, and then organic carbon was converted into organic matter as reported in Page et al., (1982).
- **Cation exchange capacity (CEC):** The Cation exchange capacity was estimated by saturate soil with (1N) pH:8.2 from sodium acetate solution, then remove sodium and extracting it with (1N) ammonium acetate solution and measuring sodium concentration in the soluble depending on flam-photometer according to Black, (1965)
- **Available Phosphorus:** was estimated by extracting with sodium bicarbonate solution (0.5 N) at pH (8.5), then the absorption was measured using Spectrophotometer at a wavelength length (880) nm depending on John, (1970) method.
- **Available potassium:** Estimated by extracting it with (1N) ammonia acetate solution (PH:7) the value of each sample for Available potassium in the solution measured by Flam-photometer, according to Page, (1982).

Table (1): Some physical and chemical properties of the study soil

Analysis	Unit	Measured
Soil Reaction (pH):	/	8.3
Electrical conductivity (EC)	dS m^{-1}	0.62
Organic matter (OM)	g kg^{-1}	49
CEC	Cmol kg^{-1}	12.46
Available P	mg kg^{-1}	27
Available K	mg kg^{-1}	21.1
Field capacity	%	24.4
Sand	g kg^{-1}	200
Silt	g kg^{-1}	274.5
Clay	g kg^{-1}	525.5
Texture	/	Silty Clay loam

Measuring phosphate enzyme activity:

Method used by Tabatabi and Bremner, (1972) have been used to measure Phosphate enzyme activity by weighing 1 gram dry soil in plastic cans and added (0.2) ml of Tolin and (4) ml buffer solution (Modified universal buffer with pH (11) and (1) ml of p-nitrophenyl phosphate in different concentrations (0.1, 0.05, 0.0125, 0.025), Molarity on soil samples, the samples were shacked for several seconds (20-25) seconds and closed after that placed in the incubator for one hour at a different temperature and added (1) ml of calcium chloride plus (4) ml of Sodium hydroxide plus (1) ml of p-nitrophenyl phosphate on the control sample were well promoted for several seconds and extract with filter paper and measured using a Spectrophotometer with a wavelength (420-400) nm.

Laboratory experiment:

Kinetic Parameters:

The kinetic parameters were studied, which include the maximum enzyme speed (Vmax) and Michaelis constant (Km) for (0-15) depth using different concentrations of the subject substrate. The enzyme effectiveness was estimated by estimating the phosphatase enzyme at each

concentration of the subject substrate Then the values of Vmax and Km were estimated according to the modified Hanes-Woolf transformation formula from Michaelis-Menten, (1913) as follows

$$\frac{s}{v} = \frac{km}{v\,max} + \frac{1}{v\,max}[S] \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where:
V = speed of reaction
Vmax = maximum speed of the enzyme
Km = Michaelis constant
S = concentration of Subject matter
Kinetic parameters Km and Vmax were extracted from the slope of the straight line equation $\frac{1}{v}\frac{1}{v}$ and the intercept representing $\frac{km}{v\,max}\frac{km}{v\,max}$. .

Statistical analysis: Complete Randomized Design (CRD) used to analyze the characteristics in three replicates using SAS contrast analysis (2010). The averages were compared using the least significant difference (L.S.D) at the level of significance (0.05) Al-Rawi and Khalaf Allah (1980).

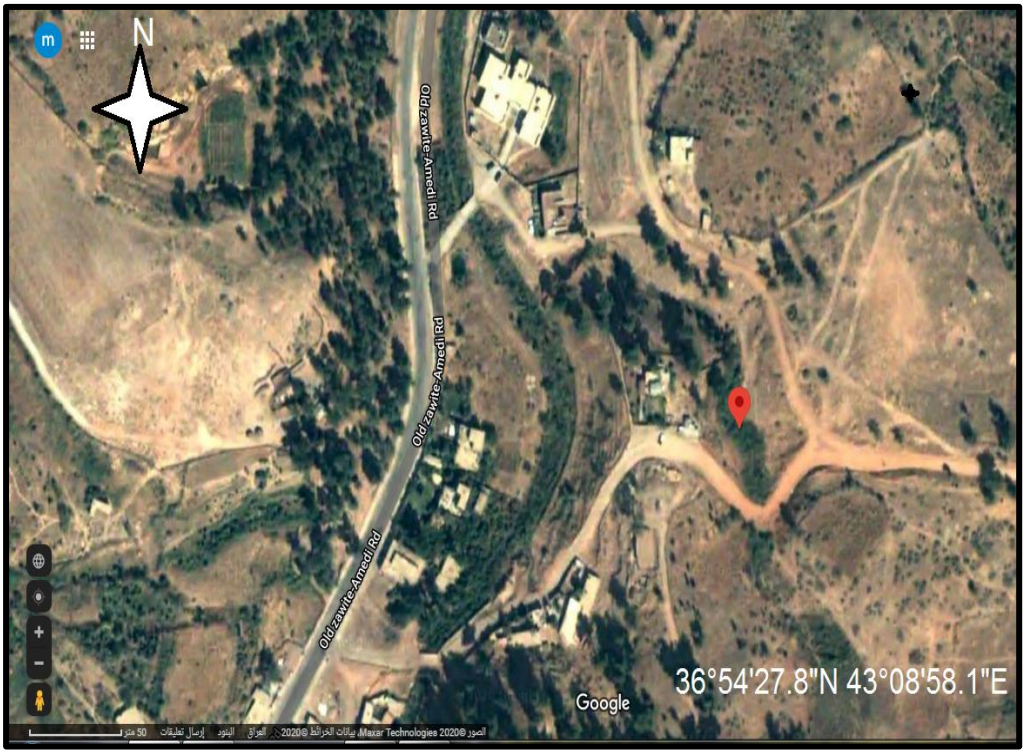


Fig (1): Sampling site

RESULTS AND DISCUSSION

Influence of temperature on phosphatase enzyme effectiveness

Figure (2) shows the increase in phosphatase enzyme effectiveness by increasing the incubation temperature. The incubation was done with a concentration (0.05 N) of the substrate, the highest value for phosphatase enzyme effectiveness reached (155.47) mg P-nitrophenol $g^{-1}Hr^{1}$, which was the result of incubating the soil at the highest temperature of the study (50) ^{o} C, the values of phosphatase effectiveness keep increasing even at the highest temperature and it gave the highest significant difference value from the rest of the temperatures. Most chemical reactions increase rapidly with an increase in temperature, as the increase in temperature gives the reacting molecules greater kinetic energy, which resulting in increased collisions per time unit.
Enzymatic reactions are affected in a similar way until the temperature reaches certain limits, then the phosphatase enzyme is negatively affected by a change in enzyme structure (Al-Jabri, 2010). These results indicate that the phosphatase enzyme effectiveness can continue to rise with a certain

temperature rise and then begin to decline, In a study for amidohydrolases enzymes Al-Jabri (2010) found an increase in the acid and alkaline phosphatase enzyme effectiveness by increasing the incubation temperature from (10 - 60) C for all the studied soils for both depths ranged from (0-15) cm to (15-30) cm, and the high incubation temperature of more than 60 °C led to a sharp significant decrease in the enzyme effectiveness. These results indicate that the alkaline phosphatase enzyme is highly active when the temperature rises above (30) C. These results are consistent with Al-Tawil, (2001); Al-Tawil, (2015); Al-Jubouri,

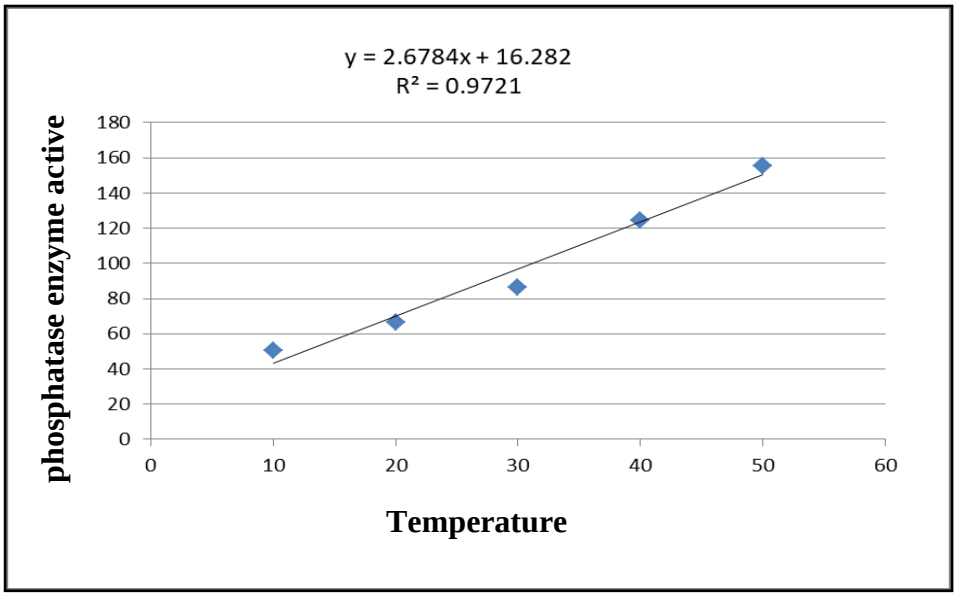


Figure (2) The relationship between temperature and phosphate enzyme effectiveness

Figure (3) shows the linear relationship between the concentration of the subject substrate p-nitrophenol [S] and alkaline phosphatase effectiveness V / [S] in the study soil according to Hanes - Woolf formula at (37) C. The results show that effectiveness of enzyme increases with the increase in the a concentrations of the substrate.

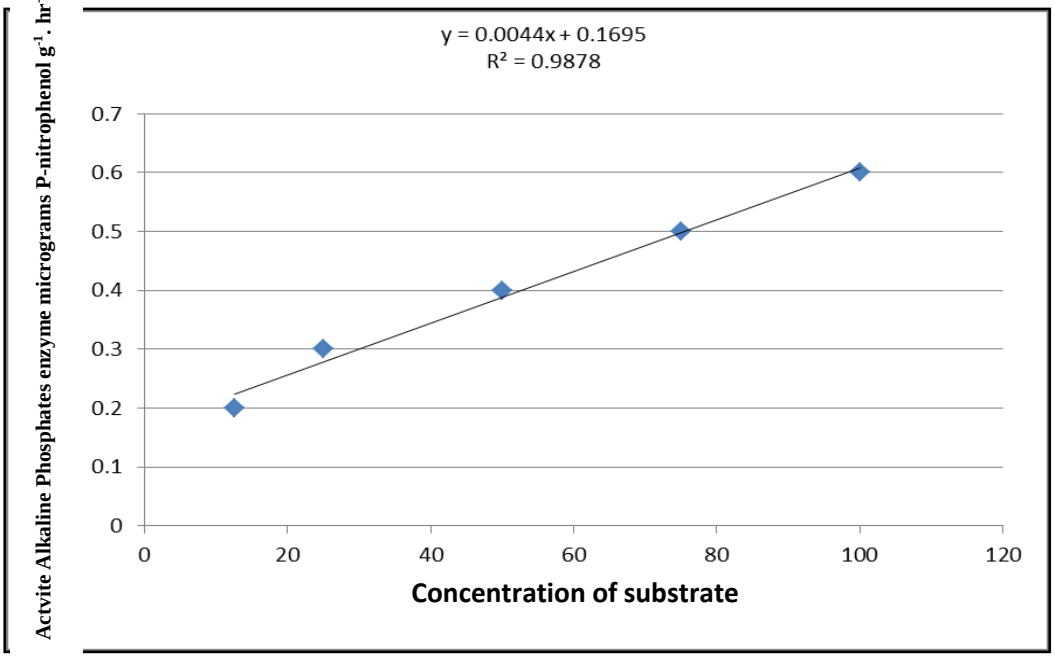


Figure (3) The relationship between the concentration of the substrate (P-nitrophenol) and phosphatase enzyme effectiveness

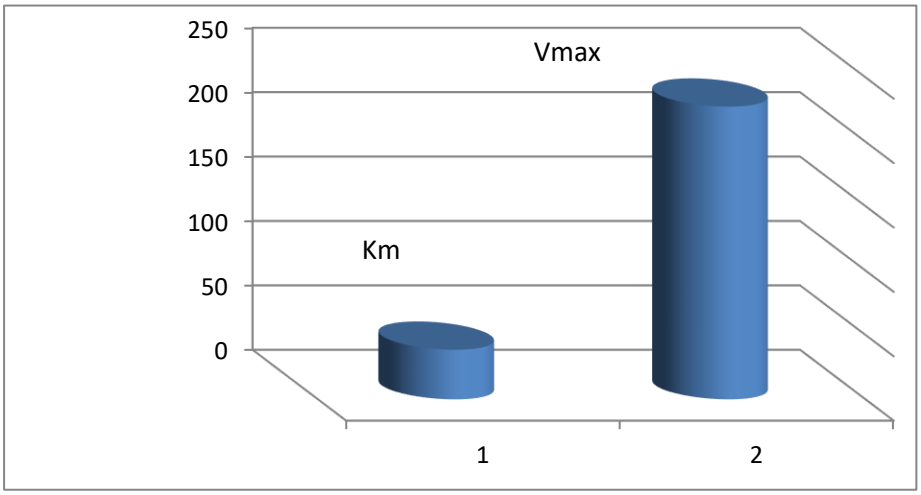
The highest value of alkaline phosphatase enzyme effectiveness (V/S) reached (0.6) micrograms P-nitrophenol. g^{-1}. hr^{-1}, which resulted from the effect of concentration (100) mMol of the subject

substrate. While the lowest value for the alkaline phosphatase enzyme effectiveness V/[S] reached (0.2) micrograms P-nitrophenol g^{-1}. hr^{-1} which resulted from the effect of the lowest substrate concentration (12.5 mM). This shows that phosphate enzyme effectiveness has not decreased even at the higher concentration indicating that the effectiveness of the phosphate enzyme can be increased in the case of using higher substrate concentrations.From the straight line equation for the relationship between the substrate concentrations and phosphate enzyme effectiveness, the value of Vmax was calculated, which is equal to 1 / slope, and the value of Km was calculated, which results from the product of multiplying the value of V max in the secant was (227.27) µg of P-nitrophenol g^{-1}. hr^{-1}.

While the Km value was (38.52) mmoler, this result was lower than the results found by Al-Taweel, (2007). In general, the Km value is an indicator of the affinity between the substrate and the enzyme, as the low Km values mean that the affinity is high between the alka;ine phosphatase enzyme and the substrate, as the enzyme needs a smaller substrate amount to reach its maximum speed, And the Km values did not take a specific direction in all soil samples of the sites, which may be due to the different sources of preparation of the enzyme, plus that these values do not depend on enzyme concentration in the soil. The low values for both field and orchard soils in different locations in Al-Diwaniyah governorate did not take a specific direction, which indicates the clear difference in enzyme processing sources and its basis is the dominant organisms on the one hand and the roots secretions on the other (Al-Jubouri, 2018).

In study about alkaline phosphatase for some soils southern Iraq for AL-Ansari et al., (1999) They found that the values of Michal's constant ranged between (0.5 - 1.4) mmol, while the maximum speed values were between (400-500) µg PNP g^{-1} soil. 1 hr^{-1}. Al-Taweel, (2001) confirmed the variation in Vmax and Km values for Imidase enzyme among the ten studied soils, as the Km values of ranged between (2.56-44.19) mmol. The Vmax values were between (12.88-446.68) µg-N NH4 gm^{-1}soil .2hr^{-1}, and found a difference in Vmax and Km values of the Amide enzyme in the rhizosphere soil of wheat and bean plants, the Vmax values were (205.88 and 216.59) Micrograms N - NH4 g^{-1} soil 2 hr^{-1} and Km values (10.78 and 9.86) mM, respectively, due to a difference in the nature of plant roots secretions, plus the difference in the numbers and types of microorganisms that secrete the enzyme. The high V max value indicates the high effectiveness of the enzyme in the soil and the difference in its sources and composition, resulting from the high amount of organic matter in the study soil and the nature of the types and numbers of microorganisms in forest soils, as well as the increase in the amount of root exudates and the type of clay minerals (Al Taweel, 2001).

Figure (4): Vmax and Km Values for phosphatase activity in zawiya soil



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تأثير تركيز المادة الخاضعة في المقاييس الحركية لأنزيم الفوسفاتيز القاعدي

مأمون شاكر زغير رند عبدالهادي غزال

قسم علوم التربة والموارد المائية ، كلية الزراعة والغابات ، جامعة الموصل

الكلمات المفتاحية:
انزيم الفوسفاتيز، المادة الخاضعة، الحركيات، زاويتا

الخلاصة

اجريت دراسة مختبرية في مختبر قسم علوم التربة والموارد المائية كلية الزراعة والغابات وفي مختبر كلية البيئة بهدف بيان نشاط وفعالية انزيم الفوسفاتيز وحساب المعايير الحركية (السرعة القصوى Vmax و وتركيز المادة الخاضعة Km) ولاجل ذلك أختيرت تربه من غابات زاويتة ذات نسجة مزيجية طينية رمليه وتحت غطاء اشجار(الصنوبر). وعوملت التربة بتراكيز مختلفة من المادة الخاضعة Substrate للانزيم وهي (0,0125 ، 0.025، 0.05، 0,075، 0,1) مولار وتحت تأثير درجات الحرارة المختلفة (10 ، 20 ، 30 ، 40 ، 50) درجة مئوية.

وأظهرت النتائج تأثير واضح للمادة الخاضعة على نشاط الانزيم إذ زادت تأثير واضح للمادة الخاضعة فعالية الانزيم مع زيادة التراكيز المضافة من المادة الخاضعة . وبلغت اعلى قيمة لفعالية أنزيم الفوسفاتيز القاعدي V /[S] (0,6) مايكرو غرام P-nitrophenol غم-1. ساعة-1 والتي نتجت عن تأثير التركيز (100) ملي مول من المادة الخاضعة. أما اقل قيمة لفعالية انزيم الفوسفاتيز القاعدي V/[S] بلغت (0,2) مايكرو غرام P-nitrophenol غم-1. ساعة-1 والتي نتجت عن تأثير أقل تركيز للمادة الخاضعة (12,5) ملي مول. وبينت النتائج أن قيمة V max بلغت (227,27) مايكرو غرام P-nitrophenol غم-1. ساعة-1. أما قيمة Km فقد بلغت (38,52) ملي مولر. أظهرت النتائج زيادة في نشاط إنزيم الفوسفاتيز بزيادة درجة حرارة الحضانة بتركيز (0.05) مولار من الركيزة ، وبلغت أعلى قيمة لنشاط إنزيم الفوسفاتيز V (155,47) ملغم P-nitrophenol غم-1 ساعة-1 ، والتي حدثت من حضانة التربة عند أعلى درجة حرارة مستخدمة في الدراسة (50) درجة مئوية ، حيث لوحظ أن قيم فعالية إنزيم الفوسفاتيز استمرت في الزيادة حتى أعلى درجة حرارة مستخدمة في الدراسة.