Original Article

Effect Of Fertilization Of Bio-Rhizobacterein, Nano-NPK And Nano-Micro Complete On The Enzymatic Activity Of Bean Rhizosphere

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Abstract

A factorial experiment was carried out in the wooden canopy of Agriculture and Forestry College / University of Mosul during the winter season on 15/11/2021, to study the effect of bio-fertilizer Rhizobacterein, Nano-NPK 20:20:20 and Nano-micro complete with the time periods on the activity of urease and phosphatase enzyme using the soil of Hawi el-mosul region with (Silt Loam) texture. The experiment included two factors, the first factor included nine treatments of Nano-NPK20:20:20, Nano-micro complete fertilizers , Rhizobacterein biofertilizer, traditional fertilizer, doul and triple treatments. The second factor included the time periods (60, 90 and 120) days after of cultivation according to the complete random design (CRD) with three replicates. The original Italian bean (Luz) seeds were planted on 24 November, 2021, in ten kg soil of plastic. Two batches of fertilizer were applied to the soil combined with germination, the first batch 20 days later, and the second batch 20 days later. For all treatments, the efficacy of each urease and phosphatase enzyme was assessed, for the planting periods (60, 80, and 120) days after cultivation.

The results of the statistical analysis of $LSD_{0.05}$ showed that the tri combination of fertilization (Nano-NPK 20:20:20 + Nano- Micro Complete + Bio- Rhizobacterein) for the periods 60, 90 and 120 days after cultivation was superior to the activity of the urease enzyme (120.00, 60.70 and 48.70) µg $N\text{-}NH_4$ gm^{-1} soil 2 hr^{-1} respectively, followed by the same combination of regular sources for the same time periods (83.30, 41.00, and 34.80) µg $N\text{-}NH_4$ gm^{-1} soil 2 hr^{-1} respectively, While the same trend was achieved with the activity of the phosphatase enzyme for the tri-nano combination (430.9, 320.1, and 277.00) µg PNP gm^{-1} soil 1 hr^{-1} respectively, compared to the conventional mixture and for the same periods (348, 235.2, and 135.00) µg PNP, 1 gm^{-1} soil 1 hr^{-1}, sequentially.

Introduction:

Biofertilizers are considered at the present time a promising alternative for the sustainability of agriculture and rationalization in adding chemical fertilizers while reducing the pollution from environmental sources , as the addition of biofertilizers to the soil or seeds pollination increases the elements availability for the plant and reduces the chemical fertilizers requirements, which improves plant growth (Rabie and Hamiany, 2004) , Biofertilizers are additives of microbiological origin that contain bacterial, fungal, or both types of organisms, particularly bacteria that promote plant development and create phytohormones. (Akbarinia et al., 2003). Bacterial biofertilizers are an abundant alternative to mineral fertilizers and reduce environmental damage as well as stimulate crop growth (Brito et al., 2018). In order to improve nutrient behavior, nano-fertilizers are a promising technology, They are chemical compounds that are produced using a variety of techniques to produce nutrient particles or its oxides that are between (1-100) nm, before being chelated, coated with polymers, or placed in polymers to release slowly, it's Characterized by a high surface area, high penetration, transport and

assimilation speed, with a high nutrient use efficiency. Given that it contains one or more plant nutrients, it might have additional components added to promote plant growth and production. (Al-Jutheri et al., 2022).

Biofertilizers are also produced by isolating, purifying and characterizing selected strains of beneficial microbectiria in the soil and multiplying them in suitable farms until they are used either by mixing with seeds before planting or contamination of the roots or directly added to the soil in order to provide the plant with nutrients such as nitrogen and phosphorus by activating them in soil or in Rhizosphere and make it available for the plant soon (Hari et al., 2010). The double application of nano- and bio-fertilizers promotes the supply of nitrogen and phosphorus, reduces environmental pollution brought on by the excessive use of chemical fertilizers, and increases crop quality. (Abd El-Aziz, et al., 2007). The Bio-fertilizers has an important role In fixing atmospheric nitrogen, symbiotic with leguminous crops, as has been proven, especially with bean crops, and non-symbiotic with non-legume crops, and also improving the nutritional status of plants through fungal and bacterial vaccenation, which lead to an increase in the efficiency of phosphorus absorption. Potassium and other elements absorption is believed to be the result of the improvement of the nutritional balance to absorb nitrogen and phosphorus and increase plant size. Because enzymes are important to the cycle of nutrients and their readiness in the soil, measurements of the activity of the soil's enzymes are significant indicators of the state of the soil's environmental conditions.

Due to their great sensitivity to these changes and availability of assessment, soil enzymes play a crucial role as an indicator of positive or negative changes in soil management. (Bandick and Dick, 1999 and Hassan, 2021). Enzymatic activity is an useful indicator of soil fertility because enzymes are important for many soil-level processes, including the decomposition of the organic fertilizers and the conversion of nutrients from their organic to their inorganic plant-available forms. This is only done by the enzymes secreted by microorganisms, especially Bacteria and fungi, the protease enzyme decomposes protein and liberates nitrogen in the form of ammonia, and the phosphatase enzyme degrades organic phosphorus such as Phytin, DNA and RNA into phosphorus available for plants.

The urease enzyme works by hydrolyzing urea that has been applied to the soil as fertilizer or by converting animal and plant waste into carbon dioxide and ammonia while slightly increasing the soil response values. (Andrews et al., 1989, Byrnes and Amberger, 1989, Al-Karaawi, 2020). The phosphatase enzyme plays a major and effective role in the soil system (Dick and Tabatabai, 1992, Dick et al., 2000, and Zheng et al., 2021), It performs the function of mineralizing organic phosphorus and converting it into the available form (Yokoyama & Kitayama, 2017 and Kumar et al., 2013).Therefore, the purpose of this study was to determine the impact of the bio-fertilizer Rhizobacterin, the balanced macroelement nano-fertilizers NPK 20:20:20 and Micro Complete, and the time period on the activity of urease and phosphatase enzymes in the Rhizosphere of bean roots.

Methods and Materials:

On 24 November, 2021, an experiment was conducted in the wooden canopy of the Agriculture and Forestry College at the University of Mosul to analyze the influence of the bio-fertilizers Rhizobacterein, Nano NPK 20:20:20, and Nano-Micro Complete fertilizers well as the time on the activity of the urease and phosphatase enzymes in the soil of the bean root rhizosphere.

The Completely Randomized Design (C.R.D) was designed included two factors, The first factor was fertilization with Bio-fertilizers Rhizobacterein and Nano NPK 20:20:20, and Nano-Micro Complete fertilizers and their combinations, in addition to the traditional combination according to Table 1. The second factor was the number of days (60, 90, and 120) days after planting the seeds. The faba bean, cultivar Luz Italian origins, was planted on November 15, 2021, on soil collected from the Al-Hawi area, which is on the right side of Mosul Governorate. It was packed in polyethylene bags of 10 kg soil, after conducting the basic operations as drying, grinding and sifting, whose characteristics are shown in Table 2.

Table (1) shows the experimental parameters, quantities of fertilizers

Tr.No	Treatments	Addition levels kg ha^{-1}	
		first addition	second addition
1	Comparison	0	0
2	NPK (20:20:20) nano-chelated fertilizer	7.5	7.5
3	Micro Complete fertilizer (8% Fe, 1.5% Zn, 1.5% Mn, 0.5% B, 0.5% Mo, and 0.5% Cu) nano-chelated	5	5
4	A specialized biofertilizer for Rhizobacterein: (Azotobacter sp., Asosprillum sp. and R. leguminosarum), 10^8 cfu g^{-1}	1.5	1.5
5	Nano-NPK + Nano-Micro Complete	5+7.5	5+7.5
6	Nano- NPK + Rhizobacterein	1.5+3.75	1.5+3.75
7	Nano-Micro Complete + Rhizobacterein	1.5+2.5	1.5+2.5
8	Nano-NPK + Nano-Micro Complete + Rhizobacterein	1.5+2.5+3.75	1.5+2.5+3.75
9	(Tron) Conventional NPK + Conventional Micro Complete + Rhizobacterein	1.5+7.5+50	1.5+7.5+50

Table (2): Shows some chemical, physical and biological properties of the study soil.

properties	Value	measruing unit
Sand	62.55	g kg^{-1}
Silt	20.25	
Clay	17.20	
Texture	Sandy loam	------
Organic mater	17.6	g kg^{-1}
carbonate minerals	71	
pH	7.3	------
Ec	2.10	dS m^{-1}
CEC	29.8	$Cmol^{+}$ kg^{-1}
available N	20.42	Mg kg^{-1}
available P	11.57	
available K	250	
Total bacterial count	$^{8}10\times3.26$	CFU

The seeds were washed several times with warm water to remove suspended materials so as not to affect the microorganisms of the biofertilizer, The seeds were soaked in water before planting for 7 hours to stimulate the germination process, 4 seeds were placed in one bag at a depth of 3 cm, The humidity level was maintained close to (75%) of the Field capacity, All service operations continued, including irrigation, hoeing, cleaning to get rid of weeds, and adding fertilizers in the required batches in the soil throughout the experiment period. A complete experiment was raised with three replications (27) experimental units of the above treatments after (60, 80 and 120) days of cultivation, which represents the stage of vegetative growth, as the Rhizosphere soil was separated from the soil far from the root according to (Rengel, 1997), and estimated the activity of urease and phosphatase enzyme.

Preparing the Rhizosphere soil for analyzes:

Soil samples were taken from the soil of the rhizosphere by lifting the whole plants with their roots from the soil and moving them carefully and quietly, After this shaking process, the remaining soil on the roots represent the rhizosphere soil. This soil was taken from the roots with a small soft brush, and kept in clean and sterile plastic bags, closed well, and then placed in the refrigerator until it is used in its chemical and biological analyzes (Rengel 1997).

Determination of enzyme activity:

Estimates of enzymatic activity for (urease) and (alkaline phosphatase) enzym were made according to (Tabatabai and Bremner, 1972), which included the following:

Evaluation of the activity of the urease enzyme:

The urease enzyme activity was measured by taking (5) g of rhizosphere soil in a (50) ml volumetric flask with (0.2) ml of toluene, which inhibits microbial growth, and (9) ml of the buffer solution THAM (Tris Hydroxymethyl Amino Methane) with pH (9) and (1) ml of a 0.2 M urea solution (substrate) at (37)°C temperature for two hours, and then (35) ml of a 2.5 Mol potassium chloride solution containing silver sulfate (Ag_2SO_4 100) mg L^{-1} inhibiting enzyme activity, then volume was completed to (50) ml of the same solution, then the ammonium ion resulting from the enzyme activity was measured using the Micro-kjeldah according to (Bremner and Edwards, 1965) that mentioned in (Black, 1965b) using Heavy MgO, receiving ammonia with boric acid (H_3BO_3), then rectification with (H_2SO_4) 0.014 mol.

Determination of alkaline phosphatase enzyme activity:

The activity of the phosphatase enzyme was estimated by incubating (1) gm of rhizosphere soil in (50) ml mlvolumetric flask with the addition toluene (0.2) ml and (4) ml of MUB (Modified Universal Buffer) with pH (11) in (1) ml from pNPP (0.05) molar (p-Nitro Phenyl Phosphate Substrate) on the soil samples and the samples were shaken for (20-25) seconds, It was sealed and then placed in an incubator for one hour at a temperature of (37) °C . After that, (1) ml (0.5 M) calcium chloride ($CaCl_2$), (4) ml of (0.5 M) of sodium hydroxide (NaOH) and (1) ml of p-Nitro phenyl phosphate were added to the The comparison sample was shaken well for several seconds and filtered using filter paper. The yellow color was measured in the filter using a spectrophotometer at a wavelength of (400- 420) nm. The method used to measure the activity of the phosphatase enzyme is indirect, as it does not measure the amount of mineral phosphorus liberated from organic phosphorus, but the amount of P-nitrophenol liberated from the addition of P-nitrophenyl phosphate, a substance to the enzyme during a certain period, so whatever the greater the substance released from the PNP indicates an increase in enzyme activity and thus an increase in the amount released from mineral phosphorus.

Results and discussion

The results in table (3)showed the effect of biofertilizing Rhizobacterein, Nano-NPK and Nano- Micro Complete on the urease enzyme activity in the rhizosphere soil of the bean after (60, 90 and 120) days of cultivation, that there was a significant difference in the activity of the urease enzyme, as treatment showed nano -Micro Complete showed a significant superiority in the value of urease enzyme activity in rhizosphere compared to the comparison treatment, as the rate of enzyme activity was (26.50, 49.40, 23.90) µg $N\text{-}NH_4$ gm^{-1}soil 2 hr^{-1} respectively according to the time period (60, 80 and 120) days comparison treatment of (20.30, 45.40 and15.30) µg $N\text{-}NH_4$ gm^{-1} soil 2 hr^{-1} according to the time period respectively, The nano-NPK also achieved a significant superiority in urease enzyme activity values compared to the nano-Micro Complete and comparison treatment, as the enzyme activity reached (34.10, 63.60 and 28.70) µg $N\text{-}NH_4$ gm^{-1} soil 2 hr^{-1} for the same period respectively, As for the Rhizobacterein biofertilization was significantly superior to the two treatments of Nano -NPK and Nano-Micro Complete, the enzymatic activity reached (36.60, 69.60 and 30.30) µg $N\text{-}NH_4$, gm^{-1} soil 2 hr^{-1} respectively according to the time period, and it seems that the combined effect of the binary combination of nano NPK + Complete nanoparticles outperformed all treatments with a single effect of fertilizers in

the values of urease enzyme activity, as they amounted to (48.00, 94.60 and 38.40) µg of N-NH_4, gm $^{-1}$soil 2 hr^{-1} according to the time period (60, 80and 120) days, respectively, As we can see from the same table, the triple combination of fertilization (NPK Nano + Micro Complete + Rhyzobacterein) for the periods (80, 90 and 120) days after planting exceeded the effectiveness of the urease enzyme (120.00, 60.70 and 48.70) µg N-NH_4 gm^{-1} soil 2 hr^{-1} in succession, followed by the same combination of normal sources and same periods (83.30, 41.00, and 34.80) µg N-NH_4 gm^{-1} soil 2 hr^{-1} respectively.

This discrepancy in urease enzyme activity may be attributed to the effect of nanofertilizers with their smart delivery, slow release and work with high efficiency in penetration into plant tissues, transport and rapid exemplification within the plant compared to conventional fertilizers (Singh et al., 2021, Al-jutheri et al., 2021 and Al-jutheri et al., 2022). The enzyme gave the highest activity during the period (90) days of cultivation, as it reached (73.99) µg N-NH_4 gm^{-1} soil 2 hr^{-1} which significantly differed at other periods up to (120) days, and its average reached (30.57) µg N- NH_4 gm^{-1} soil 2 hr^{-1} as a significant decrease was observed in urease enzyme activity with the increase of the time period. The increase in enzyme activity within (80) days due to the decomposition of the bulk of the nitrogen component of the organic matter originally present in the degradable soil, as well as the nanofertilizer containing nitrogen within (90) days and because of all the appropriate conditions for decomposition of heat and humidity, and that any increase in The source of carbon, energy and food source will lead to an increase in its biomass and will inevitably be reflected in an increase in its activity and its secretion of enzymes, and this is what happens within (90) days.

The urease enzyme effectiveness in a period of (90) days was higher than (60) days, that could be due to the increase in plant growth and roots density and the important hormones secreted by it for the reproduction of organisms and increasing their numbers, therefore the increase in biomass and the enzymes it secretes that attract microorganisms that fix nitrogen, Some organisms are exposed to death and decomposition, which leads to the spread of nitrogen compounds, which is an important element for increasing the effectiveness of the urease enzyme and increasing its quantity in the rhizosphere (Liu et al., 2005 and Al-Badiri, 2021).

The decrease in enzyme activity in the period (120) days may be due to the lack of the enzyme (Substrate), which led to a lack of carbon and energy source for the microorganisms that secrete the enzyme and thus to the death of many of them due to lack of food, and the difference in enzyme activity with the time period could be due to the lack of organic sources and the lack of availability of nutrients in the last periods of growth due to their consumption by microorganisms with nutrition and increased plant growth, which is reflected in enzymatic activity, and this is consistent with what Li et al., (2022), Those who noticed an increase in enzymatic activity for the first periods of incubation and a decrease in the last periods of incubation. As for the effect of the treble combinations of nano-NPK + nano Micro Complete + bio-fertilizer Rhyzobacterin in achieving the highest activity of the urease enzyme (76.47) µg N-NH_4 gm^{-1} soil 2 hr^{-1}, There is a large variation in the activity values of the urease enzyme with the effect of fertilization treatments and planting periods, as the values of urease activity reached (20.3 - 60.7, 45.4 -120.0 and 15.3 - 48.7) at the cultivation periods (60, 80, and 120) days, respectively. We note that there is a clear difference for the planting period on urease enzyme activity, as it was found that the highest values of urease enzyme activity were at (80) days, and the activity of the urease enzyme increased in all fertilization treatments between the period (60 and 80) days, where the highest percentage of increase for the activity of urease enzyme between a period of (60 and 90) days in the sixth treatment, where the percentage of increase was (55.61)%, while the lowest percentage of was in the third treatment, as it amounted to (46.35)%. As for the urease enzyme activity at the planting period (120) days decreased from what it was at the (80) days for all fertilization treatments, The highest percentage decrease was in the comparison treatment that amounted (66.29)%, while the lowest percentage decrease was in the third treatment that amounted to (51.61)%.

Table (3) Effect of Rhizobacterein, Nano-NPK and Nano-Micro Complete and the time period on the urease enzyme activity (µg N-NH_4 gm^{-1} soil 2 hr^{-1}) in the rhizosphere

Tr .N0	Treatments / Period day	60	90	120	Rate of Treatment effect
1	Comparison	20.30	45.40	15.30	27.00
2	NPK (20:20:20) nano-chelated fertilizer	34.10	63.60	28.70	42.13
3	Micro Complete nano-chelated	26.50	49.40	23.90	33.27
4	A specialized biofertilizer for Rhizobacterin:: (Azotobacter sp., Asosprillum sp. and R. leguminosarum), 10^8 cfu g^{-1}	36.60	69.60	30.30	45.50
5	Nano-NPK + Nano-Micro Complete	48.00	94.60	38.40	60.33
6	Nano- NPK + Rhizobacterein	33.20	74.80	29.20	45.73
7	Nano-Micro Complete + Rhizobacteria	30.30	65.20	25.80	40.43
8	Nano-NPK + Nano-Micro Complete + Rhizobacterein	60.70	120.00	48.70	76.47
9	(Tron) Conventional NPK + Conventional Micro Complete + Rhizobacterein	41.00	83.30	34.80	53.03
	Rate of effect period	36.74	73.99	30.57	
L.S.D $_{0.05}$: (Treatment): 8.509 (Period): 6.614 (treatment × Period): 6.459					

The enzymatic activity increases by adding fertilizers in the first period of time up to (90) days, then the activity decreases with the progression of time at a period of (120) days due to the oxidation of the bulk of the organic matter and the decrease in the amount of energy supplied to revive the enzyme-producing soil (Hashem, 2016). Certainly, the microbial biomass in any soil is directly proportional to the content of nutrients in that soil, and therefore its secretions of enzymes increased. With combinations of fertilizers for macro and micro nutrients, the availability of nutrients in the soil increased, which encourages plant growth, increased vegetative growth, and increased photosynthesis, thus transferring part of the secretions Photosynthesis to the root system and increasing the secretions of the root system, and providing soil microorganisms with a source of carbon and energy, and thus increasing it in the soil of the rhizosphere (Al-Yasiri, 2013). The reason for the increase may be attributed to the fact that the addition of biofertilizer in the rhizosphere has led to an increase in the number of microorganisms, and thus these organisms produce many enzymes, and the fact that the rhizosphere is an area rich in root secretions including the urease enzyme in addition to being a suitable environment for the growth of microorganisms and the increase in its growth is linked to the carbonaceous substances secreted by the roots, and some of these organisms are responsible for the secretion of urease, which leads to an increase in urease enzyme effectiveness (Yoshimune, 2010, Watt, 2009, Kaur et al., 2016).

The effect of Bio-Rhizobacterein, Nano NPK, Micro Complete fertilizers and the time period on phosphatase enzyme activity (µg PNP gm^{-1} soil hr^{-1}) in the rhizosphere

The results in Table (4) showed the effect of biofertilization Rhizobactrein, Nano NPK and Nano-Micro Complete on phosphatase enzyme activity in the rhizosphere soil of the bean after (60, 90 and 120) days of planting, that there was a significant difference in the activity of the phosphatase enzyme, as the treatment Nano-Micro Complete showed had a significant superiority in the value of phosphatase enzyme activity for the rhizosphere area, compared to other treatment, As the enzyme activity rate was (125.11, 265.7 and 96.00) µg PNP gm^{-1} soil 1 hr^{-1} respectively according to the period (60, 90 and 120) days of planting compared to the control treatment of (72.00, 155.7 and 59.00) µg PNP gm^{-1}Soil 1 hr^{-1} according to the period respectively, the Nano-NPK fertilization also achieved a significant superiority in phosphatase enzyme activity value compared to nano Micro Complete and the comparison treatment of (255.7, 319.5, 211.00) µg PNP gm^{-1} Soil 1 hr^{-1} for the same time period respectively, the biofertilization Rhizobacterein was significantly superior to the two treatments of nano-NPK and nano Micro Complete, and the enzymatic activity reached (155.2, 300.8 and 119.00) µg PNP gm-1soil 1 hr-1 respectively according to the period, and it appears that the combined effect of the binary combination Nano NPK + Micro Complete It outperformed all treatments with the dual and single effect of fertilizers in the values of phosphatase enzyme activity of (199.3, 400.3 and 86.00) µg of PNP gm-1 soil 1 hr-1

according to the period (60, 80, 120) days, respectively. As we can see from the same table, the triple combination of fertilization (Nano-NPK + Micro Complete-Nano + Bio-Rhyzobacterein) for the periods (60, 80, 120) days after planting was superior to the activity of the phosphatase enzyme (320.1, 430.9, 277.00) µg PNP gm^{-1} soil hr^{-1} respectively, followed by the same The combination of conventional sources for the same periods (235.2, 348.00, 135.00) µg PNP 1 gm^{-1} soil, 1 hr^{-1} respectively. This variation in the phosphatase enzymea activity is due to the fact that the addition of biofertilizer led to an increase in microorganisms number that secrete soil enzymes (Yoshimune, 2010), and the nanofertilizer addition led to an increase in soil enzymes effectiveness including phosphatase, due to the increase in the concentration of the (subject matter) and the activity of this enzyme has a direct effect on the mineralization process, which affects the phosphorus availability which is one of the main factors for increasing the effectiveness of the phosphatase enzyme. The enzyme gave the highest activity during the period (80) days of cultivation, as it reached (313.46) µg PNP gm^{-1} soil 1 hr^{-1} which differed significantly at other time periods up to (120) days of planting, and its average was (129.00) µg PNP gm^{-1} soil 1 hr^{-1}, Since a significant decrease was observed in phosphatase enzyme activity with an increase in the time period, the increase in the activity of the enzyme during (90) days may be the reason for the decomposition of the largest part of nitrogen component of the organic matter originally present in the degradable soil, in addition to the containment of nitrogen within the nano-fertilizer during (90) days, and because of all the relevant conditions for decomposition as heat and humidity, and that any increase in carbon source, energy and food source will lead to an increase in its biomass and will inevitably be reflected in an increase in its activity and its secretion of enzymes, and this is what happens within (90) days. The phosphatase enzyme activity in (90) days was higher than (60) days. due to the important role in influencing microorganisms by providing them with energy and some components necessary to build their bodies and carry out their activities such as the secretion of auxins, enzymes and other substances that stimulate plant growth, which It leads to an increase the roots density and their secretions (Al-Jabri, 2010).

The decrease in enzyme activity during (120) days may be due to the lack of the substance of enzyme which led to a lack of carbon and energy source of microorganisms wich secrete the enzyme and thus to the death of many of them due to lack of food, and the difference in enzyme activity with the time period could be due to the lack of organic sources and the lack of availability of nutrients in the last periods of growth due to their consumption by microorganisms, And the increase in plant growth, which is reflected on the enzymatic activity, and this is consistent with what was obtained by Li et al., (2022) who noticed an increase in enzymatic activity for the first periods of incubation and a decrease in the last periods of incubation. As for the effect of the three combinations of Nano-NPK with Micro Complete-nano fertilizer with the bio-fertilizer Rhizobacterin in achieving the highest activity of the phosphate enzyme (342.67) µg PNP gm^{-1}soil 1 hr^{-1} there is a clear change in the phosphatase enzyme activity values with the effect of fertilization treatments and cultivation periods, as the Phosphatase enzyme activity values ranged (72-320.1, 155.7-430.9, and 59-277) for each of the cultivation periods of 60, 80, and 120 days, respectively. It was found the values of the phosphatase enzyme activity increased at the cultivation period of (80) days, then decreased again at the cultivation period of (120) days. Thus, the highest values were also at (80) days for all fertilization treatments, while (80) days in the comparison treatment where increase to (53.75%), while the lowest percentage increase was in the second treatment, as it amounted to (19.96%). the phosphatase enzyme activity at the period of (120) days decreased from what it was at the cultivation period of (80) days for all fertilization treatments.

Table (4) Effect of Rhizobacterin, Nano-NPK, Nano- Micro Complete biofertilizers and the time period on phosphatase enzyme activity (µg of PNP gm^{-1} soil 1 hr^{-1}) in the Rhizosphere

Tr.No	Treatments / Period day	60	90	120	Rate of Treatments effect
1	Comparison	72.00	155.7	59.00	95.57

2	**NPK (20:20:20) nano-chelated fertilizer**	**255.7**	**319.5**	**211.00**	**262.06**
3	**Micro Complete nano-chelated**	**125.11**	**265.7**	**96.00**	**162.27**
4	**A specialized biofertilizer for Rhizobacterein: (Azotobacter sp., Asosprillum sp. and R.**	**155.2**	**300.8**	**119.00**	**191.67**
5	**Nano-NPK + Nano-Micro Complete**	**199.3**	**400.3**	**86.00**	**228.53**
6	**Nano- NPK + Rhizobacteria**	**193.3**	**318.4**	**66.00**	**192.567**
7	**Nano-Micro Complete + Rhizobacterein**	**174.00**	**281.8**	**112.00**	**189.27**
8	**Nano-NPK + Nano-Micro Complete + Rhizobacterein**	**320.1**	**430.9**	**277.00**	**342.67**
9	**(Tron) Conventional NPK + Conventional Micro Complete + Rhizobacterein**	**235.2**	**348**	**135.00**	**239.4**
	Effect of period rate	**192.21**	**313.46**	**129.00**	
L.S.D $_{0.05}$: (Treatment): 8.509 (Period): 6.614 (treatment × Period): 6.459					

The results showed that the factors affecting the enzyme effectiveness depend on the biomass and the increase in the amount of material that the enzyme works on in the soil, and this highlights the positive role of increasing the microbial addition represented by bio cells. The results of the phosphatase enzyme activity rhizosphere were lower after (60) days of germination compared to the second period (90) days for all levels. these results show that the phosphatase enzyme effectiveness depends on the biomass and its activity whenever biomass was greater by increasing the microscopic cells in the soil, the more effective of enzyme, this was observed after (90) days of germination which reflects the increase in the number of cells compared to the period (60) days as well an important factor affecting enzyme activity is the presence of the enzyme substrate, If the quantity was large, this is reflected on enzyme's effectiveness increasing, and this result was observed through the results of the comparison between nano-fertilizer and bio-fertilizer. These results are consistent with Ergasheva and Egamberdieva, (2014), who indicated that the activity of soil enzymes, including phosphatases, is significantly associated with the biological activity in the soil through the amount of carbon dioxide released.

Conclusions:

It was found that the synthetic nano-fertilizers for the macro- and microelements were working with high efficiency and slow release, which stimulated the growth of the vegetative parts and roots in a harmony with the growth stages of the plant, In combination with bio-fertilizer, there was a synergistic and harmonious enhancement of root growth, which stimulated a high efficacy of enzymes when compared to traditional fertilizers.

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