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Effect Of Vermicom Post, Phosphorous Nano-Fertilizer And Humic Acid On The Activity Of Alkaline Phosphatase Enzyme And Its Kinetic Parameters

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Abstract

A field experiment was inducted during autumn season of 2020 in one of the fields of Afak district - Al-Diwaniyah governorate / Iraq to investigate the effect of vermicompost, phosphorous nano-fertilizer and humic acid on the activity of alkaline phosphatase enzyme and its kinetic parameters at the flowering and full maturity stages of maize. Randomized Complete Blocks Design was used at three replications. The experiment included 14 treatments: {Control (C), Vermicompost at 4 ton ha^{-1}(V), phosphorous nano fertilizer at 5 and kg ha^{-1} (nP1 and nP2), humic acid at 20 and 40 kg ha^{-1} (H1 and H2), VnP1, VnP2, VH1, VH2, H1nP1, H1nP2, H2nP1 and H2nP2}. The results showed that theV treatment hadahighest mean of alkaline phosphatase activity (528.1µg P-nitrophenol g^{-1} soil $1h^{-1}$) and of V_{max}(194.8µg þ-nitrophenol g^{-1} soil $1h^{-1}$) at the flowering stage only, and the lowest means of K_m(0.2403 and 0.2367mM) at the flowering and full maturity stages respectively. The nP2 treatment achieved the highest means of alkaline phosphatase activity (690.6 and 635.2µg P-nitrophenol g^{-1} soil $1h^{-1}$)at flowering and full maturity stages respectivelyand V_{max}(187.5 µg P-nitrophenol g^{-1} soil $1h^{-1}$)at flowering stage only, whereas the nP1 treatment had the lowest means of K_m (0.2527 and 0.3087) at the flowering and full maturity stages respectively.Further, the H1 treatment gave the highest means of alkaline phosphatase activity (563.3 and 515.2µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering and full maturity stages respectively and V_{max}(194.8 µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering stage only. Further, the H1 and H2 treatment achieved the lowest means of K_m (0.2483 and 0.2690mM) at the flowering and full maturity stages respectively.The VH1 treatment had the highest means of alkaline phosphatase activity (754.5 and 736.4 µg P-nitrophenol g^{-1} soil $1h^{-1}$)at flowering and full maturity stages respectively and V_{max} (192.3 µg þ-nitrophenol g^{-1} soil $1h^{-1}$)at flowering stage only. In addition to, VH1treatment gave the lowest means of K_m (0.2177 and 0.2373 mM) at flowering and full maturity stages.

Keywords: Nano-Fertilizer, Kinetic Parameters, Phosphatase Enzyme

Introduction

The effectiveness of enzymes is a biological indicator for monitoring soil quality and plays an important role in the field of soil fertility, as it cannot be replaced by any other substance because of

its ability to dissolve and prepare nutrients that are a primary source of nutrition for soil organisms (Shulka and Varma, 2011), including alkaline phosphatase enzyme, which plays a major role in the phosphorous cycle in nature by converting organic and inorganic phosphorus into ready-to-use forms by plants, as soil phosphatase enzyme breaks the bond linking phosphorous to carbon in organic matter, which leads to the release of organically bound phosphorus (Wyszkowska and Wyszkowski, 2010). Soil microorganisms are an important source of alkaline phosphatase in soil (Tarafdar and Claassen, 1988).This enzyme contains 12-15% carbohydrates of the total weight of protein, and has a molecular weight in the range of 130,000-170000 Daltons (Kim and Wyckoff, 1990). The activity of the alkaline phosphatase enzyme increases when organic fertilizers are added to the soil (Parham et al., 2002), as organic fertilizers are a specific material for the activity of enzymes in the soil, and soils rich in organic matter are characterized by a large density of microorganisms. Vermicom post is one of the most important modern organic fertilizers, as earthworms produce it by breaking down and digesting organic waste and accelerating its decomposition and it is rich in important macro and micro nutrients that are easily soluble in water (Alkhafaage et al., 2012). Further, nano-fertilizers are nutrient carriers developed using raw materials with nano dimensions ranging from 1-100 nm. The nano-particles have a high surface area and the ability to retain an abundance of nutrients and release them slowly and steadily, which facilitates the absorption of nutrients by crops (Kothari and Wani, 2019). Humic acids are of great importance in improving the biological properties of the soil due to the changes they make, which are represented in encouraging the growth and reproduction of beneficial microorganisms in the soil. They also have a vital role in the biotic and abiotic interactions that occur in the rhizosphere of the plant, which is positively reflected in various biochemical processes, including the activity of enzymes in soil (Shah et al., 2018). Meyhew (2004) and Bukowskaand Donderski (2007) showed that humic substances increased the numbers and speed of growth of microorganisms and increased the activity of phosphatase enzymes.The values of enzyme kinetics are one of the important basic constants in enzyme chemistry, as V_{max} is the maximum activity of the enzyme and $K_m$is the concentration of the subject matter, which gives half of the maximum activity and is evidence of the affinity between the enzyme and the subject matter.Studying the kinetic parameters (V_{max} and K_m) of enzymes helps to know the affinity changes between the subject matter and the enzyme and its sensitivity to temperature, as temperature affects the movement of the enzyme and the subject matter, which is reflected in the V_{max} and K_m values. Hui et al. (2013) studied the kinetic parameters of alkaline phosphatase and the results showed significant differences in the V_{max} and $K_m$values. The aim of this study is to investigate the effect of vermicompost, phosphorous nano-fertilizer and humic acid on the activity of alkaline phosphatase enzyme and its kinetic parameters at the flowering and full maturity stages of maize.

Material and Methods

A field experiment was carried out during the autumn season of 2020 in one of the fields of Afakdistrict - Al-Diwaniyah governorate / Iraq in a soil as shows their physical and chemical properties in Table 1, to investigate the effect of vermicompost, phosphorous nano-fertilizer and humic acid on the activity of alkaline phosphatase enzyme and its kinetic parameters at the flowering and full maturity stages of maize.The experiment was carried out according to Randomized Complete Blocks Design (RCBD) at three replications. The experiment included 14 treatments (table 2).Soil management were carried out as required, the net area of sub sub plot was (3 m x 3 m) 9 m^2 which contained 6 rows, 0.50 m apart and 0.25 m within the plants.Recommended

potash fertilizer (120 Kg K ha^{-1}) as potassium sulfate (41% K) was applied at the time of planting, while the nitrogen fertilizer was applied (240 Kg N ha^{-1}) as a urea (46% N) in two equal doses (1/2 at the time of planting and 1/2 at flowering stage), whereas the humic acid and phosphorous nano-fertilizer were applied at the time of plantingaccording to treatments, while vermicompost was applied in conjunction with ureaaccording to treatments.The seeds of maize hybrid (Furat) were sown on 27 July 2020 by placing 3 seeds in the hill, and then thinning to a one plant after emergence. Crop management was carried out as needed, and the plants were harvested after the appearance of maturity signs.

Table (1): Physical and chemical properties of soil

Trait	Value	Unit
Soil texture	Clay Loam	-----
Sand	20.83	%
Loam	31.24	
Clay	47.92	
Bulk density	1.27	mg m^{-3}
pH 1:1	7.48	-----
Ec 1:1	2.69	ds m^{-1}
CEC	24.47	$Cmol_c$ Kg^{-1} soil
Ca^{2+}	8.57	$Cmol_c$ L^{-1}
Mg^{2+}	5.41	
Na^{+}	3.31	
K^{+}	1.18	
Cl^{-}	5.63	
SO_4^{2-}	2.88	
HCO_3^{-}	7.63	
CO_3^{2-}	Nill	
$CaCO_3$	231.00	g Kg^{-1} soil
O.M	8.51	%
Available N	28.05	mg Kg^{-1} Soil
Available P	14.30	
Available K	146.00	

Table (2): Experimental treatments

Symbol	Treatment
C	Control (without fertilizer)
V	Vermicompost (4 ton ha^{-1})
nP1	Phosphorous Nano Fertilizer (5 kg ha^{-1})
nP2	Phosphorous Nano Fertilizer (10 kg ha^{-1})
H1	Humic acid (20 kg ha^{-1})
H2	Humic acid (40 kg ha^{-1})
VnP1	Vermicompost (4 ton ha^{-1}) + Phosphorous Nano Fertilizer (5 kg ha^{-1})

VnP2	Vermicompost (4 ton ha^{-1}) + Phosphorous Nano Fertilizer (10 kg ha^{-1})
VH1	Vermicompost (4 ton ha^{-1}) + Humic acid (20 kg ha^{-1})
VH2	Vermicompost (4 ton ha^{-1}) + Humic acid (40 kg ha^{-1})
H1nP1	Humic acid (20 kg ha^{-1}) + Phosphorous Nano Fertilizer (5 kg ha^{-1})
H1nP2	Humic acid (20 kg ha^{-1}) + Phosphorous Nano Fertilizer (10 kg ha^{-1})
H2np1	Humic acid (40 kg ha^{-1}) + Phosphorous Nano Fertilizer (5 kg ha^{-1})
H2nP2	Humic acid (40 kg ha^{-1}) + Phosphorous Nano Fertilizer (10 kg ha^{-1})

alkaline phosphatase enzyme activity (µg P-nitrophenol g^{-1} soil $1h^{-1}$) was estimated in the rhizosphere of maize during flowering and maturity stages according to Eivaziand Tabatabai (1977), while its kinetic (K_m and V_{max}) was estimated at the flowering and maturity stages by using sevenconcentrations of P-nitrophenol(0.0125, 0.025, 0.05, 0.075, 0.1, 0.125 and 0.15) mM, and then K_m and V_{max} values were estimated according to modified Hanes-Woolf equation from Michaelis-Menten equation as follows:

$$S/V = K_m/V_{max} + 1/V_{max}\,[S]$$

As:

V = Reaction velocity

V_{max} = Maximum reaction velocity

K_m =Michaelis constant (mM)

[S] = Substrate concentration (mM)

Data were statistically analyzed according to the analysis of variance by using the Gnestat software. The Duncan's multiple range was used to comparebetween means of studied traits.

Results and Discussion

Alkaline phosphatase activity (µg þ-nitrophenol g^{-1} soil $1h^{-1}$)

The results at the table (3) show that the application of vermicom post at level 4 ton ha^{-1} (V) gave a best results of alkaline phosphatase enzyme activity (528.1µg P-nitrophenol g^{-1} soil $1h^{-1}$) compared with control treatment (C) which gave the lowest (368.2µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering stage only. The reason may beattributed to the fact that vermicom post contains a highly active biological mixture of bacteria and a large group of soil enzymes and plant nutrients, which helps to increase the effectiveness of phosphatase enzymes in the soil (Parham et al., 2002 and Al-Taweel, 2015).The results indicate that the application of phosphorous nano fertilizer at 10 Kg ha^{-1} (nP2) was significantly superior and achieved the highest means of alkaline phosphatase enzyme activity (690.6 and 635.2µg P-nitrophenol g^{-1} soil $1h^{-1}$) compared with control treatment (C) which achieved the lowest (368.2and 377.0 µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering and full maturity stagesrespectively.The reason of an increase may be due to the fact that the application of nano-phosphorous in sufficient amounts had a positive effect on the growth and development of roots that stimulate the activity of microorganisms, as well as its effective role in the formation of energy, which led to an increase the activity of the alkaline phosphatase enzyme in the soil. The application of humic acid at 20 Kg ha^{-1} (H1) was significantly superior and gave the highest means of alkaline phosphatase enzyme activity (563.3 and 515.2µg P-nitrophenol g^{-1} soil $1h^{-1}$) compared with control treatment (C) which achieved the lowest (368.2and 377.0 µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering

and full maturity stages respectively (Table 3).The reason of an increase may be attributed to the role of humic acid, which is the source of carbon, which increases the activity of microorganisms, as well as the increase in the readiness of some nutrients through chelation of some micro-elements and the formation of complex and chelating compounds, which stimulates the activity of phosphatase enzymes that make the phosphate ion free in the soil solution (Pouneva, 2005).

Table (3): Effect of vermicompost, phosphorous nano-fertilizer and humic acid on the alkaline phosphatase activity (µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering and full maturity stages

Treatment	flowering stages	full maturity stage
C	368.2 f	377.0 g
V	528.1 de	396.6fg
nP1	623.3 c	577.7 c
nP2	690.9 b	635.2 b
H1	563.3 d	515.2 d
H2	530.8 de	489.6 de
VnP1	745.2 a	687.1 a
VnP2	522.4 de	465.3 de
VH1	754.5 a	736.4 a
VH2	493.8 e	446.8 ef
H1nP1	543.0 de	458.6 de
H1nP2	531.0 de	486.8 de
H2np1	537.3 de	495.5 de
H2nP2	547.5 d	497.0 de

The results in the Table (3) reveal that the application of vermicompost at 4 ton ha^{-1} + humic acid at 20 Kg ha^{-1} (VH1) was significantly superior and had the highest means of alkaline phosphatase enzyme activity (754.5 and 736.4 µg P-nitrophenol g^{-1} soil $1h^{-1}$) without significant difference with application of vermicompost at 4 ton ha^{-1} +phosphorousnano fertilizer at a 5 Kg ha^{-1} (745.2 and 687.1 µg P-nitrophenol g^{-1} soil $1h^{-1}$) whereas the control treatment (C) had the lowest (368.2and 377.0 µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering and full maturity stages respectively. The reason of an increase may be attributed to the effective role of vermicompost in improving the biological properties of the soil, increasing the efficiency of the fertilizers, as well as its role in increasing the ability of the soil to retain moisture and nutrients, which increases soil fertility and then increases the activity of beneficial soil microorganisms such as bacteria, which in turn secrete enzymes phosphatase.

Kinetic parameters (V_{max} and K_m) of alkaline phosphataseenzyme at flowering and full maturity stages

The results in the Tables (4 and 5) indicate that the V treatment gave ahighest mean of V_{max}(194.8µg P-nitrophenol g^{-1} soil $1h^{-1}$) at the flowering stage and lowest means of K_m(0.2403 and 0.2367mM) at the flowering and full maturity stages respectivelycompared with control treatment (C) which gave the lowest mean of V_{max} (185.2µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering stage and highest means of K_m (0.4190 and 0.3973 mM)at the flowering and full maturity stages respectively. The reason may be attributed to the role of vermicompost fertilizer in increasing the activity of

alkaline phosphatase enzyme (Table 3). These results are in line with Abdulkareem et al., (2013) who confirmed that the application organic fertilizers leads to an increase the V_{max} values and a decrease K_m values. The results reveal that the nP2 treatment was significantly superior and achieved a highest mean of V_{max}(187.5µg þ-nitrophenol g^{-1} soil $1h^{-1}$) without significant different with control treatment (186.3 µg P-nitrophenol g^{-1} soil $1h^{-1}$) whilethe nP1 treatment achieved a lowest mean (174.1 µg P-nitrophenol g^{-1} soil $1h^{-1}$) at full maturity stage only. However, the nP1 treatment had the lowest means of K_m (0.2527 and 0.3087) without significant different with nP2 whereas the control treatment (C) had the highest means of K_m (0.4190 and 0.3973 mM)at the flowering and full maturity stages respectively.The application ofhumic acid at 40 Kg ha^{-1} (H2) was significantly superior and gave a highest mean of V_{max}(194.8 µg P-nitrophenol g^{-1} soil $1h^{-1}$) compared with control treatment (C) which achieveda lowest (185.2µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering stageonly (Table 4). Further, the H1 and H2 treatment achieved the lowest means of K_m (0.2483 and 0.2690mM) without significant difference between them while the control treatment (C) had the highest means of K_m (0.4190 and 0.3973 mM)at the flowering and full maturity stages respectively (Table 5).The reason may be attributed to the role of humic acid in improving soil porosity and increasing its ability to retain appropriate moisture content, as there is a positive relationship between the effectiveness of phosphatase enzyme and soil porosity.The phosphatase enzyme is stabilized by organic colloids and trapped by humic acid molecules, which plays an important role in increasing the numbers of microorganisms thus increasing the activity of alkaline phosphatase (Burns, 1982).The results in the Table (4) show that the VH1 treatment was significantly superior and gavea highest mean of V_{max}(192.3µg P-nitrophenol g^{-1} soil $1h^{-1}$)compared with H1nP1, H1nP2, H2nP1, H2nP2treatments as well as VH2 treatment which gave a lowest mean (184.1 µg P-nitrophenol g^{-1} soil $1h^{-1}$) without significant difference with control treatment (C) (185.2 µg P-nitrophenol g^{-1} soil $1h^{-1}$) at flowering stage only. Regarding the K_m values,the VH1treatment gave the lowest means of K_m (0.2177 and 0.2373 mM) compared with control treatment (C) at flowering stage andH2nP2 treatment at full maturity stage which gave the highest means of K_m (0.4190 and 0.5220 mM) respectively.The superiority of VH1 in V_{max} may be attributed to the increased activity of alkaline phosphatase enzyme (Table 3).

Table (4): Effect of vermicompost, phosphorousnano-fertilizer and humic acid on the V_{max}(µg P-nitrophenol g^{-1} soil $1h^{-1}$) of alkaline phosphatase at flowering and full maturity stages

Treatment	flowering stages	full maturity stage
C	185.2ef	186.3 a
V	194.8 a	192.3 a
nP1	185.2ef	174.1 b
nP2	185.2ef	187.5 a
H1	191.0bc	192.3 a
H2	194.8 a	192.3 a
VnP1	189.9bcd	187.5 a
VnP2	189.9bcd	192.3 a
VH1	192.3ab	192.3 a
VH2	184.1 f	191.1 a
H1nP1	188.7 cd	188.7 a

H1nP2	187.5 de	188.7 a
H2np1	188.7 cd	189.9 a
H2nP2	188.7 cd	191.1 a

Table (5): Effect of vermicompost, phosphorous nano-fertilizer and humic acid on the K_m((mM) of alkaline phosphatase at flowering and full maturity stages

Treatment	flowering stages	full maturity stage
C	0.4190 a	0.3973 c
V	0.2403fg	0.2367 g
nP1	0.2527defg	0.3087ef
nP2	0.2650cdef	0.3123ef
H1	0.2483efg	0.2753fg
H2	0.2600cdef	0.2690fg
VnP1	0.3167 b	0.4433 b
VnP2	0.3103 b	0.3717 cd
VH1	0.2177 g	0.2373 g
VH2	0.2820bcdef	0.3720 cd
H1nP1	0.3020bc	0.3517cde
H1nP2	0.2810bcdef	0.3327 de
H2np1	0.2953bcd	0.4937 a
H2nP2	0.2893bcde	0.5220 a

It is noted from Figures (1 and 2) that the increase the concentration of the subject matter (P-nitrophenol) at the flowering and full maturity stages led to an increase the rate of its degradation (increasing the activity of the alkaline phosphatase enzyme) for the two stages respectively.Also, the rate of degradation of the subject matter (P-nitrophenol) differed between the studied treatments.

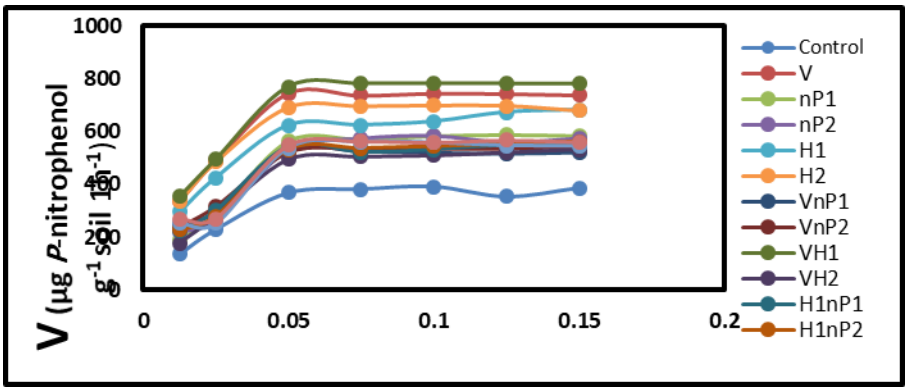

Figure (1): Relationship between the concentrations of P-nitrophenol [S] and its degradation rate (V) for the studied treatments in the alkaline phosphatase enzyme at the flowering stage

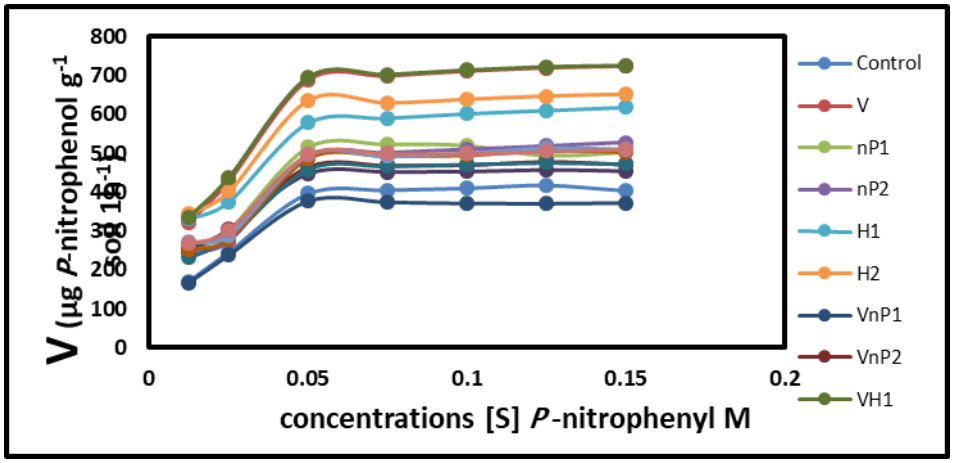

Figure (2): Relationship between the concentrations of P-nitrophenol [S] and its degradation rate (V) for the studied treatments in the alkaline phosphatase enzyme at the full maturity stage

Conclusion

We can conclude that the application ofVermicompost at 4 ton ha^{-1},phosphorous nano fertilizer at 10 kg ha^{-1}or humic acid at 20 kg ha^{-1} led to improve alkaline phosphatase activityand kinetic parameters at flowering and full maturity stages respectively, i.e.increased the V_{max}at flowering stageonlyand reduced the $K_m$at flowering and full maturity stages, while the application of phosphorous nano fertilizer at 5kg ha^{-1}led to reducing the $K_m$at flowering and full maturity stages respectively. Also, the application of Vermicompost at 4 ton ha^{-1} + humic acid at 20 kg ha^{-1} achieved the best results of alkaline phosphatase activityat flowering and full maturity stages respectively and V_{max}at flowering stage and the reduced the K_m at flowering and full maturity stages.

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