Asian Journal of Water, Environment and Pollution, Vol. 20, No. 2 (2023), pp. 31-40.
DOI 10.3233/AJW230022

Vermicompost, Phosphorous Nano-Fertiliser and Humic Acid: Their Effect on the Activity of Inorganic Pyrophosphatase Enzyme and its Thermodynamic Parameters

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Received February 10, 2022; revised and accepted January 25, 2023

Abstract: A field experiment was conducted during the autumn season of 2020 in one of the fields of Afak district - Al-Diwaniyah governorate / Iraq to investigate the effect of vermicompost, phosphorous nano-fertiliser and humic acid on the activity of inorganic pyrophosphatase enzyme and its thermodynamic parameters at the flowering and full maturity stages of maize. Randomized Complete Blocks Design was used at three replications. The experimental treatments were 14 treatments, including the control treatment and these treatments consisted of Vermicompost, Humic acid and Phosphorus and Nano-Fertiliser. The results showed that the V treatment had the highest means of inorganic pyrophosphatase enzyme activity (152.5 and 186.9 μg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) and the lowest means of Ea (10.34 and 4.72 Kj $mole^{-1}$) and Q_{10} (1.162-1.123 and 1.071-1.054) at the flowering and full maturity stages, respectively. The nP_2 treatment achieved the highest means of inorganic pyrophosphatase enzyme activity (71.6 and 55.9 μg PO_4^{-3}- P g^{-1} soil $5h^{-1}$) at the flowering and full maturity stages, respectively, and the lowest means of Ea (3.54 Kj $mole^{-1}$) and Q_{10} (1.053-1.041) at the full maturity stage only. Further, the H_1 treatment gave the highest means of inorganic pyrophosphatase enzyme activity (64.5 and 75.9 μg *P*-nitrophenol g^{-1} soil $1h^{-1}$) and the lowest means of Ea (8.31 and 3.17 Kj $mole^{-1}$) and Q_{10} (1.128-1.097 and 1.043-1.033) at flowering and full maturity stages, respectively. Also, the VnP_1 and VH_1 treatments gave the highest means of inorganic pyrophosphatase enzyme activity (159.0 and 222.3 μg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) for both treatments at flowering and maturity stages, respectively, while the H_1nP_1 and Vnp_1 treatments gave the lowest means of Ea (3.72 and 1.59 Kj $mole^{-1}$) and Q_{10} (1.056-1.043 and 1.023-1.018) for both treatments at flowering and maturity stages, respectively.

Key words: Inorganic pyrophosphatase, thermodynamic, enzyme activity.

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 e inorganic pyrophosphatase enzyme, which plays a major role in the breakdown of Pyrophosphate to Orthophosphate (Tabatabai, 1994). The activity of the inorganic pyrophosphatase enzyme increases in the surface layer of the soil, and it has a significant positive relationship with the organic carbon and the clay percentage, and a negative relationship with the calcium carbonate percentage in the soil surface (Tabatabai and

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Dick, 1979). The decomposition of pyrophosphate is affected by several factors such as temperature increase, hydrogen ion concentration increase, enzymatic activity, the ionic environment in solution and other factors such as moisture content, soil texture, soil organic matter content and its structural properties such as particle size and composition. Vermicompost is one of the most important modern organic fertilisers, produced by earthworms by breaking down and digesting organic waste and accelerating its decomposition, it is rich in important macro and micro nutrients that are easily soluble in water (Al-Khafaage et al., 2012; Al-Taweel and Al- Budairy, 2021). Further, nano-fertilisers are nutrient carriers developed using raw materials with nano dimensions ranging from 1 to 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, by 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). Thermodynamic parameters are the important indicators to know the extent of enzyme stability and efficiency in enzymatic reactions when changing temperatures, as the temperature coefficient (Q_{10}) represents a guide to knowing the kinetic energy needed by enzymatic reactions, while the activation energy (Ea) reflects the affinity between the enzyme and the subject substance and the strength of formation the complex intermediate consisting of the enzyme and the substrate (Sherene, 2017). The values of thermodynamic constants are affected by the conditions surrounding the enzyme, which depend on the soil properties and its management, as the application of fertilisers of various types has a role in improving the biochemical properties of the soil, due to its contribution to encouraging the growth and reproduction of microorganisms, and then increasing the number of secreted enzymes, improving their behaviour and characteristics, including inorganic pyrophosphatase enzyme. Therefore, this study aims to investigate the effect of vermicompost, phosphorous nano-fertiliser and humic acid on the activity of inorganic pyrophosphatase enzyme and its thermodynamic 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 Afak district-Al-Diwaniyah governorate/Iraq in soil as shown in their physical and chemical properties in Table 1, to investigate the effect of vermicompost, phosphorous nano-fertiliser and humic acid on the activity of inorganic pyrophosphatase enzyme and its thermodynamic parameters at the flowering and full maturity stages of maize.

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	
$SO4^{2-}$	2.88	
HCO_3^{-}	7.63	
$CO3^{2-}$	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	

The experiment was carried out according to Randomized Complete Blocks Design (RCBD) at three replications. The experiment included 14 treatments (Table 2). Soil management was carried out as required, and the net area of experimental unit was (3 m × 3 m) 9 m^2 which contained 6 rows, 0.50 m apart and 0.25 m within the plants.

Recommended potash fertiliser (120 Kg K ha^{-1}) as potassium sulphate (41% K) was applied at the time of planting, while the nitrogen fertiliser was applied (240 Kg N ha^{-1}) as 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-fertiliser were applied at the time of planting according to treatments, while vermicompost was applied in conjunction with urea according to treatments. The seeds of maize hybrid (Furat) were sown on 27 July 2020 by placing 3 seeds on the hill and then thinning them to one plant after emergence. Crop management was carried out as needed, and the plants were harvested after the appearance of maturity signs.

Table 2: Experimental treatments

Symbol	*Treatment*
C	Control (without fertiliser)
V	Vermicompost (4 ton ha^{-1})
nP_1	Phosphorous Nano Fertiliser (5 kg ha^{-1})
nP_2	Phosphorous Nano Fertiliser (10 kg ha^{-1})
H_1	Humic acid (20 kg ha^{-1})
H_2	Humic acid (40 kg ha^{-1})
VnP_1	Vermicompost (4 ton ha^{-1}) + Phosphorous Nano Fertiliser (5 kg ha^{-1})
VnP_2	Vermicompost (4 ton ha^{-1}) + Phosphorous Nano Fertiliser (10 kg ha^{-1})
VH_1	Vermicompost (4 ton ha^{-1}) + Humic acid (20 kg ha^{-1})
VH_2	Vermicompost (4 ton ha^{-1}) + Humic acid (40 kg ha^{-1})
H_1nP_1	Humic acid (20 kg ha^{-1}) + Phosphorous Nano Fertiliser (5 kg ha^{-1})
H_1nP_2	Humic acid (20 kg ha^{-1}) + Phosphorous Nano Fertiliser (10 kg ha^{-1})
H_2nP_1	Humic acid (40 kg ha^{-1}) + Phosphorous Nano Fertiliser (5 kg ha^{-1})
H_2nP_2	Humic acid (40 kg ha^{-1}) + Phosphorous Nano Fertiliser (10 kg ha^{-1})

Studied Traits

1. Inorganic pyrophosphatase enzyme activity was estimated in the rhizosphere of maize during the flowering and maturity stages according to Dick and Tabatabai (1978).
2. Thermodynamic parameters of inorganic pyrophosphatase enzyme (Ea and Q_{10}) were estimated at the flowering and maturity stages at six temperatures (10ºC, 20ºC, 30ºC, 40ºC, 50ºC and 60ºC) using the following equations:

A. Energy of Activation (Ea)

Log K = (- Ea / 2.303 RT) + Log A (Tabatabai, 1994)

As: A = Preexponential factor

Ea = Energy of activation (KJ $mole^{-1}$)R = 8.314 j $degree^{-1}$ $mole^{-1}$

T = Temperature (Kalvin)

The Ea values were calculated by plotting the linear relationship between Log K (the logarithm of efficiency) and 1/T (the reciprocal of temperature) for the temperatures that caused the increase in activity by extracting the slope of the straight line equal to -Ea/ 2.303 R.

B. Temperature Coefficient (Q_{10})

Q_{10} = exp. [10000 Ea / 8.314 T (T + 10)]

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

Results and Discussion

Inorganic Pyrophosphatase Enzyme Activity ($\mu g\ PO_4^{-3}$–P g^{-1} soil $5h^{-1}$)

The results in Table 3 show that the application of vermicompost at 4 ton ha^{-1}(V) achieved the best results of inorganic pyrophosphatase enzyme activity (152.5 and 186.9 µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) compared with control treatment (C) which achieved the lowest (59.4 and 48.3 µg PO_4^{-3}-P g^{-1} soil 5 h^{-1}) at flowering and maturity stages, respectively. The reason for an increase may be due to the role of vermicompost in preparing microorganisms with carbon and other nutrients necessary for their growth, increasing their activity and sustaining their metabolic activities, including the secretion of enzymes, as well as its role in protecting the enzyme from the degradation processes that it is exposed to by the proteinase enzyme that analyses the protein of the enzyme. Jain et al. (2016) reported that the enzymes adsorbed on the organic matter surface, so the amount of enzymes increases with the increase in the addition of the organic matter this is consistent with the study of Al-Taweel and Al-Budairy (2021). The results indicate that the application of phosphorous

Table 3: Effect of vermicompost, phosphorous nano-fertiliser and humic acid on the inorganic pyrophosphatase enzyme activity (µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) at flowering and full maturity stages

Treatment	*Flowering stages*	*Full maturity stage*
C	59.4 d	48.3 g
V	152.5 a	186.9 b
nP_1	68.3 cd	55.5 fg
nP_2	71.6 cd	55.9 fg
H_1	64.5 d	56.6 fg
H_2	75.9 cd	70.4 ef
VnP_1	159.0 a	157.6 c
VnP_2	113.5 b	109.4 d
VH_1	154.8 a	222.3 a
VH_2	120.1 b	186.0 b
H_1nP_1	70.2 cd	64.7 fg
H_1nP_2	70.5 cd	60.1 fg
H_1nP_1	80.0 cd	70.9 ef
H_2nP_2	98.8 bc	86.3 e

nano fertiliser at 10 Kg ha^{-1} (nP_2) was significantly superior and achieved the highest means of inorganic pyrophosphatase enzyme activity (71.6 and 55.9 µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) with a non-significant difference with nP1 treatment whereas the control treatment (C) achieved the lowest (59.4 and 48.3 µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) at flowering and maturity stages respectively. The reason for an increase may be due to the positive role of the application of nano-phosphorous in sufficient amounts in 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 inorganic pyrophosphatase enzyme in the soil. The application of humic acid at 20 Kg ha^{-1} (H_1) was significantly superior and had the highest means of inorganic pyrophosphatase enzyme activity (64.5 and 75.9 µg *P*-nitrophenol g^{-1} soil $1h^{-1}$) compared with control treatment (C) which had the lowest (59.4 and 48.3 µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) at flowering and maturity stages, respectively (Table 3). The reason for the increase may be attributed to the role of humic acid in improving the physical, chemical and biological properties of soil, which was positively reflected in the increase in the activity of the inorganic pyrophosphatase enzyme (Pouneva, 2005). The results in the Table 3 reveals that the VnP_1 and VH_1 treatments were significantly superior and gave the highest means of inorganic pyrophosphatase enzyme activity (159.0 and 222.3 µg PO_4^{-3}-P g^{-1} soil 5 h^{-1}) for both treatments at flowering and maturity stages respectively compared with control treatment (C) which had the lowest (59.4 and 48.3 µg PO_4^{-3}-P g^{-1} soil 5 h^{-1}) at flowering and maturity stages, respectively.

Energy of Activation (Ea) and Temperature (Q_{10}) of Inorganic Pyrophosphatase Enzyme

The values of Ea were determined by the linear relationship shown in Figures 1-4 and the straight line equation shown in Tables 4 and 5 from the negative slope of the straight line. As for the Q_{10} values, they indicate the effect of interaction between temperature change from 10°C to 60°C for the study parameters on the activity of the inorganic pyrophosphatase enzyme for the flowering and full maturity stages. The results in Figures 5 and 6 show that increasing the incubation temperature from 10°C to 60°C affected the inorganic pyrophosphatase enzyme activity at the flowering and full maturity stages, as the increase in temperature led to an increase in the enzyme activity up to the maximum of 50°C, then the increase of temperature to 60°C led to a significant decrease in the activity of the inorganic pyrophosphatase enzyme. Also, the results in Tables 6-8 indicate that the application of vermicompost at 4 ton

Table 4: Correlation coefficient values and linear equations of the calculating of the thermodynamic parameters of the inorganic pyrophosphatase enzyme at the flowering stage

Treatment	*r value*	*Linear equations*
Control	0.720	y = -0.8352x + 4.3038
V	0.802	y = -0.5409x + 3.8042
nP_1	0.838	y = -0.7864x + 4.2437
nP_2	0.819	y = -0.8236x + 4.345
H_1	0.804	y = -0.4334x + 3.1331
H_2	0.860	y = -0.5346x + 3.4964
VnP_1	0.715	y = -0.3145x + 3.1504
VnP_2	0.866	y = -0.5621x + 3.8886
VH_1	0.816	y = -0.557x + 3.9134
VH_2	0.926	y = -0.3981x + 3.3444
H_1nP_1	0.546	y = -0.1943x + 2.4296
H_1nP_2	0.456	y = -0.2197x + 2.4929
H_2nP_1	0.754	y = -0.4622x + 3.253
H_2nP_2	0.673	y = -0.4657x + 3.2605

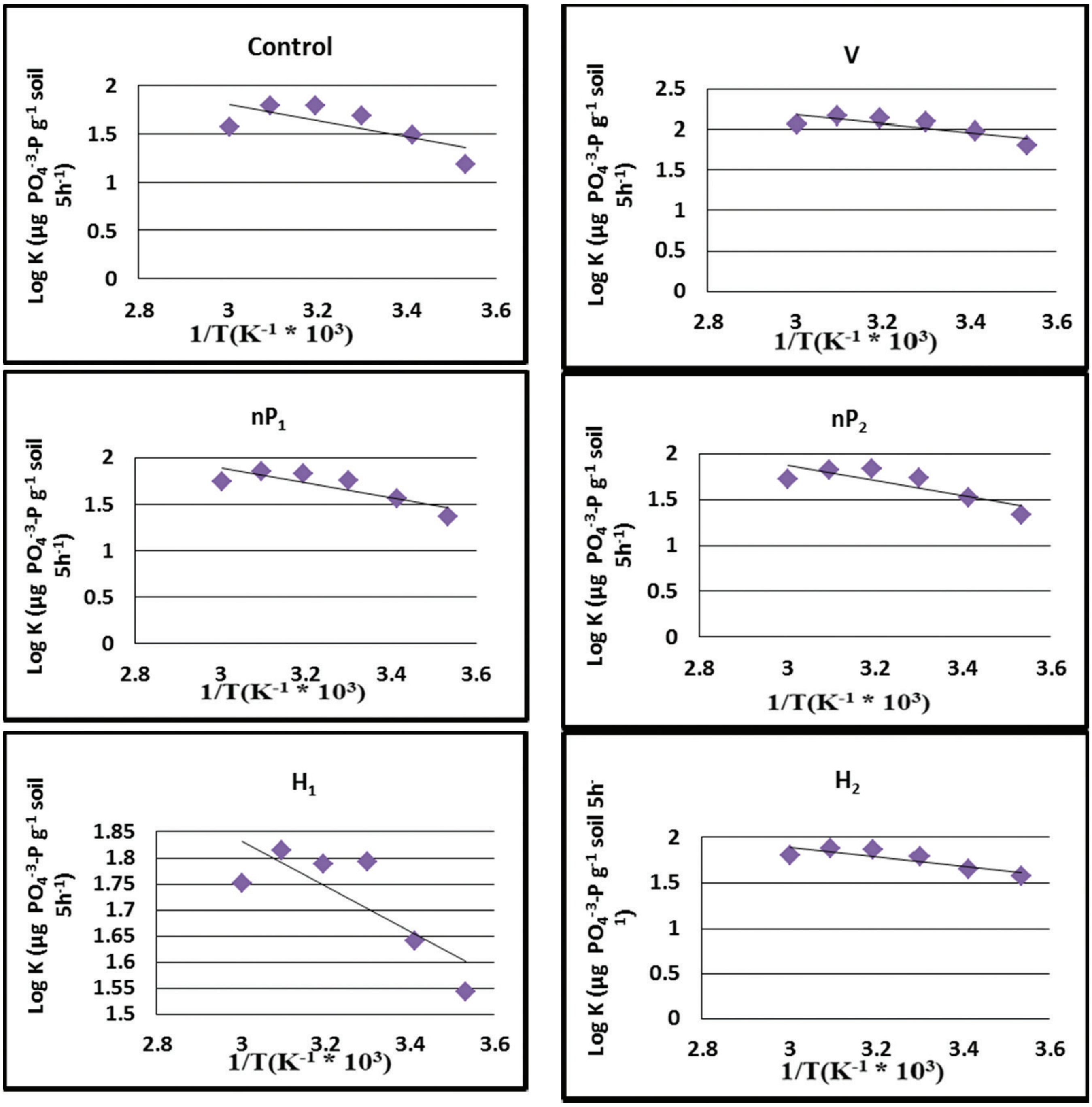

Figure 1: The relationship between 1/T and Log K (µg PO_4^{-3}–Pg^{-1} soil 5 h^{-1}) for the study coefficients at the flowering stage.

ha^{-1} (V) led to a significant decrease of Ea (10.34 and 4.72 Kj $mole^{-1}$) and Q_{10} (1.162-1.123 and 1.071-1.054) compared with control treatment (C) which achieved the highest means of Ea (16.37 and 12.58 Kj $mole^{-1}$) and Q_{10} (1.270-1.202 and 1.201-1.151) at flowering and maturity stages, respectively. The reason for the decrease may be attributed to the role of the application of vermicompost in improving the physical properties of the soil and increasing the soil's ability to retain water for a longer period. Also, the highest decomposition of the subject matter occurred at 45-50°C, as the decomposition of pyrophosphate increases with an increased moisture content of the soil and temperature (Ahmad and Kelso, 2001).

The results in Tables 6-8 reveal that there was no significant difference between the application of

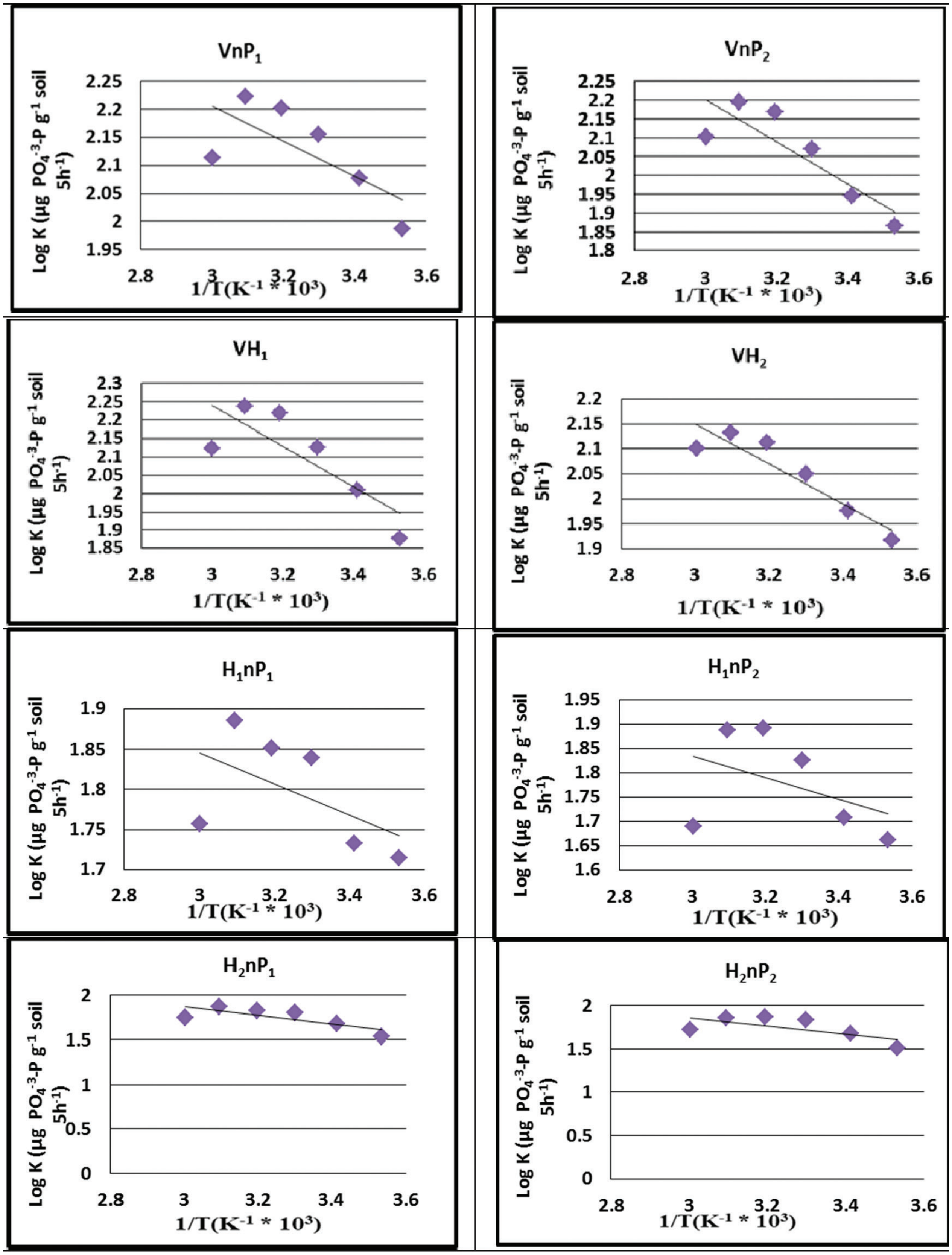

Figure 2: The relationship between 1/T and Log K (μg PO_4^{-3}–Pg^{-1} soil 5 h^{-1}) for the study coefficients at the flowering stage.

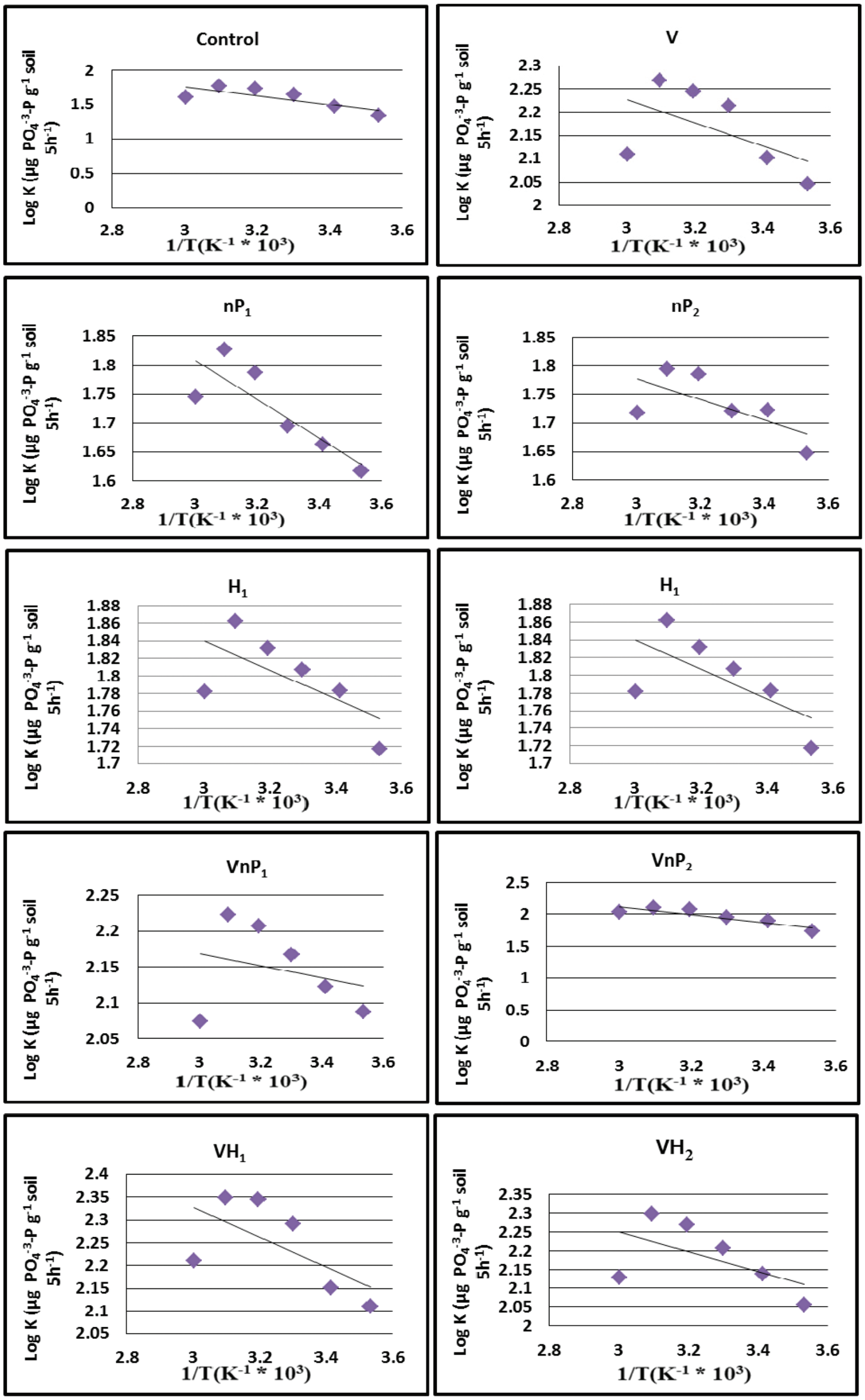

Figure 3: The relationship between 1/T and Log K (µg PO_4^{-3}–Pg^{-1} soil 5 h^{-1}) for the study coefficients at the full maturity stage.

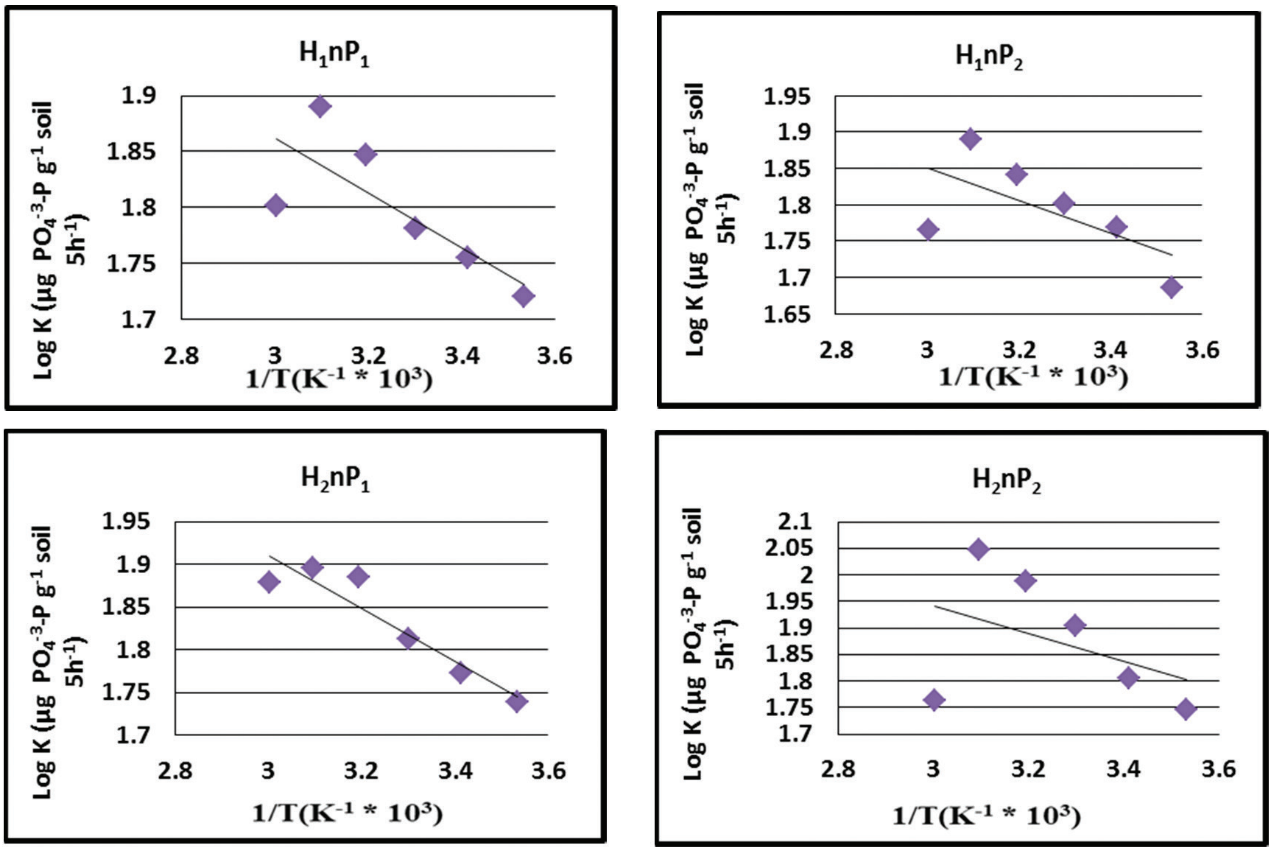

Figure 4: The relationship between 1/T and Log K (µg PO_4^{-3}–P g^{-1} soil $5h^{-1}$) for the study coefficients at the full maturity stage.

Table 5: Correlation coefficient values and linear equations of the calculating of the thermodynamic parameters of the inorganic pyrophosphatase enzyme at thefull maturity stage

Treatment	*r value*	*Linear equations*
Control	0.792	y = -0.6511x + 3.718
V	0.538	y = -0.2465x + 2.9665
nP_1	0.847	y = -0.3365x + 2.8181
nP_2	0.662	y = -0.1782x + 2.3116
H_1	0.660	y = -0.1658x + 2.337
H_2	0.450	y = -0.1513x + 2.3364
VnP_1	0.269	y = -0.0842x + 2.4213
VnP_2	0.892	y = -0.6143x + 3.962
VH_1	0.642	y = -0.3263x + 3.306
VH_2	0.566	y = -0.2626x + 3.0382
H_1nP_1	0.781	y = -0.2434x + 2.5922
H_1nP_2	0.638	y = -0.2256x + 2.5271
H_2nP_1	0.939	y = -0.3105x + 2.842
H_2nP_2	0.419	y = -0.2628x + 2.7305

phosphorous nano fertiliser treatment (nP_1 and nP_3) and controltreatment in Ea and Q_{10} values at the flowering stage only, while the application of phosphorous nano fertiliser at 10 Kg ha^{-1} (nP_2) led to a significant decrease of Ea (3.54Kj $mole^{-1}$) and Q_{10} (1.053-1.041) compared with control treatment (C) which had the highest value of Ea (12.58 Kj $mole^{-1}$) and Q_{10} (1.201-1.151) at full maturity stage. The results in Tables 6-8 show that the application of humic acid at 20 Kg ha^{-1} (H_1) led to a significant decrease of Ea (8.31 and 3.17 Kj $mole^{-1}$) and Q_{10} (1.128-1.097 and 1.043-1.033) compared with control treatment (C) which achieved the highest means of Ea (16.37 and 12.58 Kj $mole^{-1}$) and Q_{10} (1.270-1.202 and 1.201-1.151) at flowering and maturity stages, respectively. The application of H_1nP_1 and Vnp_1 treatments led to a significant decrease of Ea (3.72 and 1.59 Kj $mole^{-1}$) and Q_{10} (1.056-1.043 and 1.023-1.018) for both treatments at flowering and maturity stages, respectively, whereas the control treatment (C) gave the highest means of Ea (16.37 and 12.58 Kj $mole^{-1}$) and Q_{10} (1.270-1.202 and 1.201-1.151) at flowering and maturity stages, respectively (Tables 6, 7 and 8).

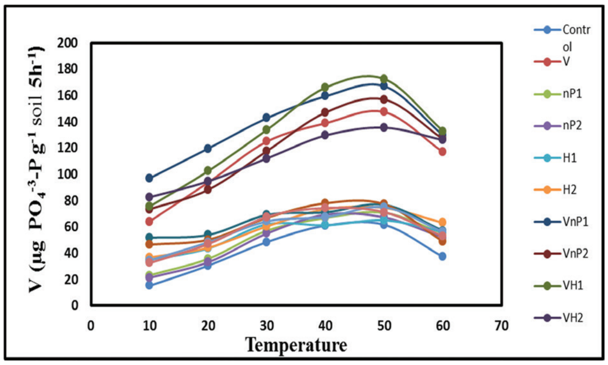

Figure 5: The relationship between temperatures and the activity of inorganic pyrophosphatase (V) for the study treatment at the flowering stage.

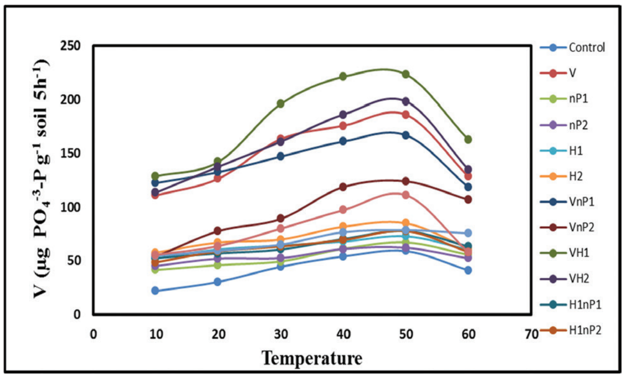

Figure 6: The relationship between temperatures and the activity of inorganic pyrophosphatase (V) for the study treatment at the full maturity stage.

Table 6: Effect of vermicompost, phosphorous nano-fertiliser and humic acid on Ea (Kj mole^{-1}) at flowering and full maturity stages

Treatment	*Flowering stages*	*Full maturity stage*
C	16.37 a	12.58 a
V	10.34 b	4.72 bcd
nP_1	15.04 a	6.71 b
nP_2	15.81 a	3.54 cde
H_1	8.31 bc	3.17 cde
H_2	10.27 b	2.88 de
VnP_1	6.03 cd	1.59 e
VnP_2	10.75 b	11.77 a
VH_1	10.84 b	6.20 bc
VH_2	7.82 bc	5.04 bcd
H_1nP_1	3.72 d	4.65 bcd
H_1nP_2	4.25 d	4.36 bcd
H_2nP_1	8.85 bc	5.66 bcd
H_2nP_2	8.92 bc	5.04 bcd

Table 7: Effect of vermicompost, phosphorous nano-fertiliser and humic acid on Q_{10} at flowering stage

Treatment	*Q_{10}*				
	10-20	*20-30*	*30-40*	*40-50*	*50-60*
C	1.270 a	1.250 a	1.232 a	1.216 a	1.202 a
V	1.162 b	1.150 b	1.140 b	1.131 b	1.123 b
nP_1	1.244 a	1.226 a	1.210 a	1.196 a	1.183 a
nP_2	1.258 a	1.239 a	1.222 a	1.207 a	1.193 a
H_1	1.128 bc	1.119 bc	1.111 bc	1.104 bc	1.097 bc
H_2	1.161 b	1.149 b	1.139 b	1.130 b	1.122 b
VnP_1	1.092 cd	1.085 cd	1.080 cd	1.074 cd	1.070 cd
VnP_2	1.169 b	1.157 b	1.146 b	1.137 b	1.128 b
VH_1	1.171 b	1.158 b	1.148 b	1.138 b	1.129 b
VH_2	1.121 bc	1.112 bc	1.105 bc	1.098 bc	1.092 bc
H_1nP_1	1.056 d	1.052 d	1.048 d	1.045 d	1.043 d
H_1nP_2	1.064 d	1.059 d	1.056 d	1.054 d	1.049 d
H_2nP_1	1.137 bc	1.128 bc	1.119 bc	1.111 bc	1.104 bc
H_2nP_2	1.138 bc	1.129 bc	1.120 bc	1.112 bc	1.105 bc

Table 8: Effect of vermicompost, phosphorous nano-fertilizer and humic acid on Q_{10} at full maturity stage

Treatment	*Q_{10}*				
	10-20	*20-30*	*30-40*	*40-50*	*50-60*
C	1.201 a	1.186 a	1.173 a	1.162 a	1.151 a
V	1.071 bcde	1.066 bcde	1.062 bcde	1.058 bcde	1.054 bcde
nP_1	1.103 b	1.096 b	1.089 b	1.084 b	1.078 b
nP_2	1.053 cde	1.050 cde	1.046 cde	1.043 cde	1.041 cde
H_1	1.047 cde	1.044 cde	1.041 cde	1.038 cde	1.036 cde
H_2	1.043 de	1.040 de	1.037 de	1.035 de	1.033 de
VnP_1	1.023 e	1.022 e	1.020 e	1.019 e	1.018 e
VnP_2	1.186 a	1.173 a	1.161 a	1.150 a	1.141 a
VH_1	1.094 bc	1.088 bc	1.082 bc	1.077 bc	1.072 bc
VH_2	1.076 bcd	1.071 bcd	1.066 bcd	1.062 bcd	1.058 bcd
H_1nP_1	1.070 bcde	1.065 bcde	1.061 bcd	1.060 bcd	1.053 bcd
H_1nP_2	1.065 bcde	1.061 bcde	1.057 bcd	1.053 bcd	1.050 bcd
H_2nP_1	1.086 bcd	1.080 bcd	1.075 bcde	1.070 bcde	1.066 bcde
H_2nP_2	1.076 bcd	1.071 bcd	1.066 bcde	1.062 bcde	1.058 bcde

References

Ahmad, F. and W.L. Kelso (2001). Pyrophosphate as a source of phosphorus: Hydrolysis under different conditions. *Journal of Research (Science)*, **(12):** 2130-2139.

Al-Khafaage, N.F., AL-Obaidi, A.A. and S.A.A. L-Shadeedi (2012). Effect of earthworms coelomic fluid treating on seed germination of cress (*Lepidium sativum*) and radish (*Raphanus saltivus*). *Asian JOF Plant Science and Research*, **2(4):** 337-383.

Al-Taweel, L.S. and Z.J. Al-Budairy (2021). Influence of vermicompost, seaweed extract and nitrogen fertilisers on maize (*Zea mays* L.) soil rhizosphere microbes. *Asian Journal of Water, Environment and Pollution*, **18(3):** 379-385.

Dick, W.A. and M.A. Tabatabai (1978). Inorganic pyrophosphatase activity of soils. *Soil Biol. Biochem*., 1**0:** 59-65.

Kothari, R. and K.A. Wani (2019). Environmentally friendly slow release nano- chemicals in agriculture: A synoptic review. *In:* Smart Farming Technologies for Sustainable Agricultural Development (pp. 220-240). IGI Global.

Jain, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D. and Y. Luo (2016). Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis*, Soil Biol. Biochem*., **101:** 32-43.

Pouneva, I. (2005). Effects of humic substances on the growth of microalgal cultures. *Russian J. of Plant Physiology*, **52(3):** 410-413.

Shulka, G. and A. Varma (2011). Soil Biology. *In:* Soil Enzymology (Vol. 22). Springer-Verlag Berlin Heidelberg.

Tabatabai, M.A. (1994). Soil enzyme. *In:* Weaver, R.W., Angle, S., Bottomyley, P., Bezdicek, D., Smith, S., Tabatabai, M.A. and A. Wollum (eds). Methods of soil analysis: Part 2. Microbiological and Biochemical Properties. Soil Science Society of America Inc., Wiley: USA.

Tabatabai, M.A. and W.A. Dick (1979). Distribution and stability of pyrophosphate in soils. *Soil Biology and Biochemistry*, **11:** 655-659.

Shah, Z.H., Rehman, H.M., Akhtar, T., Assamadany, H., Hamooh, B.T., Mujtaba, T., Daur, I., Al Zahrani, Y., Al Zahrani, H.A.S., Ali, S.,Yang, S.H. and G. Chung (2018). Humic substances: Determining potential molecular regulatory processes in plants. *Frontiers in Plant Science*, **263(9):** 1-12 .

Sherene, T. (2017). Role of soil enzymes in nutrient transformation*. Bio Bulletin*, **3(1):** 109-131.