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Effect of Vermicompost, Seaweed Extracts and Nitrogen Fertilizers on L-Asparatase Enzyme Activity and its Kinetic

Luma Salih Jabbar Al-Taweel and Zahraa Jassim Kadhum Al-Budairy
Dept. of Soil Sciences and Water Resourses, College of Agriculture, Al-Qadisiyah University, Iraq
Emails: Luma.altaweel@qu.edu.iq and Zzaah94@gmail.com

ABSTRACT

To study the effect of application of vermicompost, seaweed extracts and nitrogen fertilizers on the activity of the amidohydrolases enzymes (L-Aspartase), their kinetic measures in the rhizosphere at the flowering and maturity stages of *Zea mays* L. hybrid Furat a field experiment was carried out during the autumn season of 2019-2020 in one of the fields of Al-Noria village - Al-Diwaniyah governorate / Iraq. The experiment was arrangement as factorial according to randomized complete blocks design (RCBD) at three replications. The experiment included three factors, the first factor included the application of vermicompost at three levels (0, 2 and 4 ton ha^{-1}) symbol as A0, A1 and A2 respectively, while the second factor included application of seaweed extract at two levels (0 and 40 Kg ha^{-1}) symbol as B0 and B1 respectively, whereas the third factor included application three levels of nitrogen fertilizer (0, 120 and 240 kg N ha^{-1}) symbol as C0, C1 and C2 respectively. The application of vermicompost at level 2 ton ha^{-1} (A1) was significantly superior in L-Aspartase enzyme activity at the flowering and maturity stages and K_m and V_{max} of L-Aspartase enzyme at the maturity stage (136.7 and 156.5 µg N-NH_4^+ g^{-1} soil $24h^{-1}$, 12.57 mM, 44.8 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) respectively, while the application of vermicompost at level 4 ton ha^{-1} (A2) gave the highest means of K_m and V_{max} of L-Aspartase enzyme at the flowering stage (38.90 mM, 409.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) respectively. Also, The application of seaweed extracts at level 40 Kg ha^{-1} (B1) was significantly superior in L-Aspartase enzyme activity and V_{max} of L-Aspartase enzyme at the flowering and maturity stages (122.00 and 143.00 µg N-NH_4^+ g^{-1} soil $24h^{-1}$, 352.0 and 39.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) respectively, and K_m of L-Aspartase enzyme at the flowering stage (38.70 mM). The application of nitrogen fertilizer at level 120 Kg N ha^{-1} (C1) was significantly superior in L-Aspartase enzymes activity at the flowering stage (123.3 µg N-NH_4^+ g^{-1} soil $24h^{-1}$). The interaction between vermicompost and seaweed extracts had significant effect on the L-Asparatase enzyme activity at the flowering and maturity stages and K_m and V_{max} at the maturity stage, while the interaction between vermicompost and nitrogen fertilizer had significant effect on the L-Asparaginase enzymes activity at the flowering and maturity stages and K_m and V_{max} at the maturity stage, whereas the interaction between seaweed extract and nitrogen fertilizer had significant effect on the L-Asparaginase enzymes activity at the flowering and maturity stages and K_m and V_{max} at the maturity stage. From other hand. The interaction between three factors were significantly effect on the L-Aspartase enzyme activity at flowering and maturity stages and V_{max} at maturity stage.

Key words: Amidohydrolases enzyme, rhizosphere, V_{max}, K_m, Maize

INTRODUCTION

Soil is one of the natural ingredients in agriculture, and its quality and fertility plays an important role in the productivity of various plants, despite its importance, but Iraqi soils, especially in the central and southern regions, are still suffering from low readiness of many nutrients necessary for plant growth and development, which is due to many reasons, including it is related with soil

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characteristics such as the degree of interaction, texture and composition, as well as its low content of organic matter and others related with agricultural intensification and failure to follow the scientific method in soil nutrient management programs, which requires work to apply appropriate fertilizer programs for each soil that may lead to an improvement the soil's physical, chemical and biological properties, and as a result an increase Its fertility (Colombo et al., 2002).

The application of nitrogen fertilizers is one of the factors that increase soil fertility, and it is one of the major elements necessary for the plant due to its physiological roles within the plant tissue, except that the excessive use of nitrogen fertilizers is a result of its loss from soils by volatilization and leaching (Bronson, 2004), as well as unexamined applications to nitrogen fertilizer leads to an imbalance in the readiness of the elements in the soil, which negatively affected in the soil fertility (Havlin et al., 2005).

To reduce these negative impacts, modern trends of agriculture emphasize reducing pollutants and replacing mineral fertilization or part of it with organic fertilizers, such as the application of seaweed extracts, which is one of the most important biological fertilizers due to its content of organic matter that retains moisture and helps increase the readiness of nutrients (Spinelli et al., 2009), as well as their content of many natural compounds that act as growth regulators (O′Dell, 2003), and their role in improving the physical properties of the soil as they help in improving the conditions of ventilation, permeability, water holding, reducing soil bulk density and increasing its porosity (Naseem, 2005). The application of mineral fertilizers can also be reduced by applying other types of organic fertilizers known as vermicompost, which is an organic fertilizer that earthworms produce by analyzing organic compounds and converting them to the simple substances that benefit the plant, in addition to, the studies indicated that the vermicompost contains a number of enzymes such as peroxidase, protease and Amylase that enhance the efficacy of soil microorganisms (Bottinellin et al., 2010).

The following of the correct approach to soil service, especially the integration between the use of mineral and organic fertilizers, is the key to managing successful operations as a result of their effect on the chemical and physical properties of the soil as well as their effect on the activity of micro-organisms and the enzymatic activity in the soil, which is a biological indicator for monitoring soil quality through its participation in metabolic processes. (Yang et al., 2008). One of these enzymes is L-Asparatase, which is one of the amidohydrolases enzymes that contribute to the decomposition of organic matter as a result of its role in adding water and converting it into simpler compounds (Blank, 2004). This enzyme converts aspartic acid into ammonia and fumaric acid. When ammonia increases, aspartic acid is formed, which is included in the citric acid cycle. So, the increase in the activity of this enzyme increases the mineralization of organic nitrogen in the soil (Snewo and Tabatabai, 1996), while the kinetic measures of the enzyme reflect the maximum velocity (V_{max}) of the maximum activity of the enzyme, and the Michaelis constant (K_m) reflects the affinity between the enzyme and the subject matter. Therefore, the present study aims to study the effect of application of vermicompost, seaweed extracts and nitrogen fertilizers as well as the interaction between them on the activity of the L-Aspartase enzyme and their kinetic measures in the rhizosphere of maize at the flowering and maturity periods.

MATERIAL AND METHODS

A field experiment was carried out during the autumn season of 2019-2020 in one of the fields of Al-Noria village - Al-Diwaniyah governorate / Iraq in in a soil as shows their physical and chemical properties in Table 1, to study the effect of vermicompost, seaweed extract and nitrogen mineral fertilizer as well as the interaction between them on the activity of the L-Asparagenase enzyme and its kinetic measures in the rhizosphere of maize at the flowering and maturity periods. The experiment was arrangement as factorial experiment according to Randomized Complete Blocks Design (RCBD) at three replications. The experiment included three factors, the first factor included the application of vermicompost at three levels (0, 2 and 4 ton ha^{-1}), the physical and chemical properties of vermicompost shows in Table 2, while the second factor included application of seaweed extract at to levels (0 and 40 Kg ha^{-1}), whereas the third factor included application three levels of nitrogen fertilizer (0, 120 and 240 kg N ha^{-1}).

Table (1): Physical and chemical properties of soil

Trait	Value	Unit
Sand	217	g Kg^{-1} soil
Loam	368	
Clay	415	
Bulk density	1.33	mg m^{-3}
pH 1:1	7.69	-----
Ec 1:1	2.53	ds m^{-1}
CEC	22.13	$Cmol_c$ Kg^{-1} soil
Ca^{2+}	15.24	$Cmol_c$ L^{-1}
Mg^{2+}	10.43	
Na^{+}	22.20	
K^{+}	1.58	
Cl^{-}	23.00	
SO_4^{2-}	15.10	
HCO_3^{-}	19.00	
CO_3^{2-}	Nill	
$CaCO_3$	255.00	g Kg^{-1} soil
O.M	4.65	%
Total nitrogen	0.40	
Available N	26.11	mg Kg^{-1} Soil
Available P	13.10	
Available K	137.00	
L-Asparatase activity	25.60	µg N-NH_4^{+} g^{-1} soil 2 h^{-1}

Soil management especially plowing were carried out as required, the net area of sub sub plot was (2 m long x 2 m width) 4 m^2 which contained 4 rows, 0.50 m apart and 0.25 m within the plants. Recommended phosphorus fertilizer (100 Kg P ha^{-1}) as super triphosphate (48% P_2O_5) and potash fertilizer (120 Kg K ha^{-1}) as a potassium sulfate (41.5% K) were applied at the time of planting, while the vermicompost and nitrogen fertilizers (as a urea 46% N) were applied according to treatments in two equal doses (1/2 at the time of planting and 1/2 at flowering stage), whereas the seaweed extracts was applied in four equal doses according to growth season peroid. The seeds of maize hybrid (Furat) were sown on 27 July 2019 by placing 3 seeds in the hill, and then thinning to a one plant after emergence. Crop management were carried out as needed, and the plants were harvested after the appearance of maturity signs.

Table (2): Physical and chemical properties of vermicompost

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Trait	Value	Unit
Moisture	26.40	%
Ec 1:5	1.68	ds m^{-1}
pH	6.20	-----
O.M	43.24	%
O.C	25.14	
N	1.50	
C:N ratio	16.76	-----
Fe	0.11	mg Kg^{-1} Soil
Mn	0.68	
Zn	0.05	
B	0.29	
Cu	0.33	
Co	0.04	
Ni	0.04	

L-Asparatase enzyme activity (µg N-NH_4^+ g^{-1} soil $24h^{-1}$) was estimated in the rhizosphere of maize during flowering and maturity stages according to Senow and Tabatabai (1996), while its kinetic (K_m and V_{max}) was estimated at the flowering and maturity stages by using eight concentrations of L- aspartic acid (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40) mM according to Senow and Tabatabai (1996) which reported in Hofstee (1952), 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)

The recorded data were statistically analyzed according to the analysis of variance by using the Gnestat software. The least significant difference (LSD) was used to compare calculated average of studied traits (Steel and Torrie, 1980).

Results and Methods

L-Asparatase enzyme activity (µg N-NH_4^+ g^{-1} soil $24h^{-1}$)

The results at the tables 3 and 4 show that the application of vermicompost at level had 2 ton ha^{-1} (A1) gave the best results of L-Asparatase enzyme activity (136.7 and 156.5 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared without application of vermicompost which gave the lowest (71.0 and 74.3 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The reason of increasing may be due to the physical and chemical properties of vermicompost (Table 2), or may be attributed to role of organic matter application in increasing the number of micro-organisms, which in turn increases the production of multiple enzymes, including L-aspartase, as well as the importance of roots and their secretion that attract micro-organisms that produced the hydrolysis enzymes (Sinsabaugh et al., 2005). L-Asparatase enzyme activity was significantly affected by application of seaweed extracts (B1) and recorded the highest means (122.0 and 143.2 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with control (B0) which recorded the lowest (104.1 and 111.3 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at

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flowering and maturity stages respectively. The reason of an increasing may be due to the positive effect of seaweed extracts in increasing the growth and secretion of roots and increasing microorganisms in the soil (Coelho et al., 2016). The application of 120 Kg N ha^{-1} (C1) was significantly superior and gave the highest means of L-Asparatase enzyme activity (123.3 and 131.1 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) while the control (C0) gave the lowest (97.1 and 124.7 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The reason of the an increase may be attributed to the role of nitrogen fertilizer in changing the physical, chemical and biological properties of soil and increasing the activity of the microorganisms responsible for the decomposition of organic matter and the positive reflection of that in an increasing the secreted enzymes such as amidohydrolases enzymes (Bergstrom et al., 1998; Al-Taweel and Abo-Tabikh, 2019).

Table (3): Effect of vermicompost, seaweed extracts and nitrogen fertilizer on L-Asparatase enzyme activity (µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at floweing stage

Vermicompost levels (ton ha^{-1})		**A0**	**A1**	**A2**
		71.0	136.7	131.6
LSD .05		**5.3**		
Seaweed extracts (Kg ha^{-1})		**B0**	**B1**	
		104.1	122.0	
LSD .05		**4.3**		
Nitrogen levels (Kg N ha^{-1})		**C0**	**C1**	**C2**
		97.1	123.3	118.9
LSD .05		**5.3**		
Interaction A x B				
		B0	**B1**	
A0		51.8	90.2	
A1		123.3	150.1	
A2		137.4	125.7	
LSD 0.05		**7.5**		
Interaction A x C				
		C0	**C1**	**C2**
A0		50.1	68.0	94.9
A1		121.3	154.8	134.0
A2		119.8	147.1	127.8
LSD 0.05		**9.2**		
Interaction B x C				
		C0	**C1**	**C2**
B0		84.7	114.6	113.2
B1		109.5	132.0	124.6
LSD 0.05		**7.5**		
Interaction A x B x C				
		C0	**C1**	**C2**
A0	**B0**	33.1	41.8	80.4
	B1	67.1	94.2	109.4
A1	**B0**	98.6	142.4	128.8
	B1	144.1	167.2	139.1
A2	**B0**	122.4	159.5	132.1
	B1	117.2	134.6	110.2
LSD 0.05		**13.0**		

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Table (4): Effect of vermicompost, seaweed extracts and nitrogen fertilizer on L-Asparatase enzyme activity (µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at maturity stage

Vermicompost levels (ton ha^{-1})		**A0**	**A1**	**A2**
		74.3	156.5	151.0
LSD .05		**6.5**		
Seaweed extracts (Kg ha^{-1})		**B0**	**B1**	
		111.3	143.2	
LSD .05		**5.2**		
Nitrogen levels (Kg N ha^{-1})		**C0**	**C1**	**C2**
		124.7	131.1	125.7
LSD .05		**N.S**		
Interaction A x B				
		B0	**B1**	
A0		36.0	112.6	
A1		139.7	173.2	
A2		158.3	143.7	
LSD 0.05		**9.2**		
Interaction A x C				
		C0	**C1**	**C2**
A0		65.7	72.5	84.6
A1		154.9	162.0	152.6
A2		153.5	159.5	139.9
LSD 0.05		**11.3**		
Interaction B x C				
		C0	**C1**	**C2**
B0		101.0	121.6	111.3
B1		148.4	141.0	140.0
LSD 0.05		**9.2**		
Interaction A x B x C				
		C0	**C1**	**C2**
A0	**B0**	29.6	34.3	43.9
	B1	101.9	110.0	125.3
A1	**B0**	120.6	152.5	146.1
	B1	189.2	171.4	159.1
A2	**B0**	152.8	177.9	144.1
	B1	154.3	141.1	135.6
LSD 0.05		**15.9**		

The interaction between vermicompost and seaweed extract had significant effect on the L-Asparatase enzyme activity, the A1B1 had the highest values (150.1 and 173.2 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with A0B0 (50.1 and 65.7 µg N-NH_4^+ g^{-1} soil $2h^{-1}$) at flowering and maturity stages respectively. Also, The interaction between vermicompost and nitrogen fertilizer levels had significant effect on the L-Asparatase enzyme activity, the A1C1 recorded the highest values (154.8 and 162.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) while the A0C0 recorded the lowest (51.8 and 36.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The reason may be due to the role of vermicompost in increasing the organic carbon in the soil, which is the main energy source for micro-organisms, and the role of urea in providing a nitrogen source needed by the micro-organisms, which led to an increase the biomass at rhizosphere and increase the activity of the microorganisms which secreted enzymes (Aeschbacher and Schwarzenbach, 2010). The interaction

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between application of seaweed extracts and nitrogen fertilizer levels had significant effect on the L-Asparatase enzyme activity, the B1C1 and B1C0 gave the highest values (132.0 and 141.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with B0C0 which gave the lowest (84.7 and 101.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The results at the tables 3 and 4 reveal that the interaction between three factors had significant effect on the L-Asparatase enzyme activity, the A1B1C1 and A1B1C0 had the highest values (167.2 and 189.2 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) whereas the A0B0C0 had the lowest (33.1 and 29.6 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The results show that the L-Asparatase enzyme activity values at the maturity stage were higher than of flowering stage when application of vermicompost and seaweed extracts with nitrogen fertilizer. The reason may be due to the cumulative effect of the applied organic fertilizers in improving the physical, chemical and biological properties of the soil, as well as the role of urea in providing a nitrogen source needed by the micro-organisms and then increasing the activity of the biomass which positively reflected on the an increase of secreted enzymes, including L-Asparatase (Sinsabaugh et al., 2005).

K_m (mM) and V_{max} (µg N-NH_4^+ g^{-1} soil $24h^{-1}$)

The results at the tables 5, 6, 7 and 8 indicate that the application of vermicompost at level had 4 ton ha^{-1} (A2) gave the best results of K_m and V_{max} of L-Asparatase enzyme (38.90 mM and 409.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at the flowering stage, while at maturity stage the A1 gave the highest means (12.57 mM and 44.8 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with control (A0) which gave the lowest (24.80 and 7.55 mM and 135.0 and 25.7 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The reason may be attributed to the fact that the organic matter is gradually being mined by the microorganisms present in the rhizosphere, and the total nitrogen percentage increases after a period, which leads to an increase the numbers of micro-organisms and increase in their activity, which causes increase the maximum speed of the enzymes (Geisseler and Scow, 2014). The application of seaweed extracts (B1) was significantly affected on the K_m (38.70 mM) at flowering stage only and V_{max} (352.0 and 39.0 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively compared with control (B0) which recorded the lowest mean of K_m (26.3 mM) at flowering stage and lowest means of V_{max} (198.0 and 33.3 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering and maturity stages respectively. The reason of the superiority of seaweed extracts may be due to the importance of organic matter as a source of energy, carbon and other nutrients that micro-organisms need to grow and increase their activity, which helps them to perpetuate their metabolic activities, including their secretion of enzymes, as well as it plays an important role, which is protecting the enzyme from the degradation processes by the proteinase enzyme (Jian et al., 2016). Senow and Tabatabai (1996) reported that the V_{max} value, which represents the maximum average activity of the enzyme, is obtained when all the enzyme active sites are completely saturated with substrate. The levels of nitrogen fertilizer were non-significant difference in K_m and V_{max} values of L-Asparatase enzyme at flowering and maturity stages. The interaction between vermicompost and seaweed extracts had significant effect on the K_m and V_{max} values at maturity stage only (Tables 5, 6, 7 and 8). The A1B0 had the highest value of K_m (14.76 mM) and the A1B1 had the highest value of V_{max} (52.1 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with A0B0 which had lowest (6.02 mM and 20.1 µg N-NH_4^+ g^{-1} soil $24h^{-1}$). The reason of significant interaction at maturity stage compared with flowering stage may be due to the slow decomposition of organic fertilizers (Havlin et al., 2005). Regarding the interaction between vermicompost and nitrogen fertilizer levels had significant effect on the K_m and V_{max} values at maturity stage only, the A1C1 gave the highest values (16.03 mM and

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57.4 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) whereas the A0C1 gave lowest (4.51 mM and 16.9 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) respectively. The interaction between application of seaweed extracts and nitrogen fertilizer levels had significant effect on the K_m and V_{max} values at maturity stage, the B1C1 recorded the highest values (12.79 mM and 45.7 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with B1C0 and B0C1 which recorded the lowest (5.10 mM and 27.9 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) respectively. The reason of increasing may be due to the ability of seaweed extracts was applied with nitrogen fertilizer in increase the biological activity at the rhizosphere as result to the increase in the number of microorganisms, which leads to an increase the maximum activity of enzymes. The results at the tables 5, 6, 7 and 8 indicate that the interaction between three factors had non-significant effect on the K_m values at flowering and maturity stages and V_{max} values at flowering stage only. However, the A1B1C1 gave the highest value (59.7 µg N-NH_4^+ g^{-1} soil $24h^{-1}$) compared with A0B0C1 which gave the lowest (5.1 µg N-NH_4^+ g^{-1} soil $24h^{-1}$). The relationship between the concentrations of the substrate (L-asparatic acid) and the velocity of its degradation for the study treatments is a positive relationship, as Figures 1 and 2 show that an increase in the concentration of the substrate led to an increase the velocity of its degradation (L-Asparatase enzyme activity) at the flowering and maturity stages respectively to a certain extent and after that any increase in the concentration of the substrate was not accompanied by an increase in the activity of the enzymes, as the reaction follows the reaction of the first order to the point of the concentration at which the maximum activity (V_{max}), followed by the reaction after the order of zero. This may be due to the fact that the substrate molecules may combine with the enzyme to the extent that these sites are saturated and then the activity of the enzyme remains constant, such that any increase in the concentration of the substrate does not lead to an increase in the activity (Senow and Tabatabai, 1996). It is also noted from Figures 1 and 2 that the rate of degradation of the substrate differed between the study treatments because vermicompost has a role in increasing the readiness of nutrients and then increasing the absorption of NPK, which is positively reflected on plant growth and then the increase in the activity and growth of the root system and the increase in biomass activity. Regarding the seaweed extracts, its contain some vitamins, hormones and amino acids which plays the role of the plant growth and then increase the effectiveness of the roots and the activity of microorganisms (Sunarpi et al 2010). In the same direction, Burns (1986) explained that the higher K_m value, the affinity constant between the enzyme and the substrate, the weaker the affinity between the enzyme and substrate and this is due to the strong blocking of the enzyme by the soil components.

Table (5): Effect of vermicompost, seaweed extracts and nitrogen fertilizer on K_m of L-Asparatase enzyme activity (mM) at flowering stage

Vermicompost levels (ton ha^{-1})	**A0**	**A1**	**A2**
	24.80	33.70	38.90
LSD .05	**6.90**		
Seaweed extracts (Kg ha^{-1})	**B0**	**B1**	
	26.30	38.70	
LSD .05	**5.70**		
Nitrogen levels (Kg N ha^{-1})	**C0**	**C1**	**C2**
	31.60	34.40	35.50
LSD .05	**N.S**		
Interaction A x B			
	B0	**B1**	

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A0		16.40	33.30	
A1		31.00	36.50	
A2		31.70	46.20	
LSD 0.05		**N.S**		
Interaction A x C				
		C0	**C1**	**C2**
A0		22.40	23.90	28.00
A1		31.20	42.60	27.20
A2		40.90	36.50	39.40
LSD 0.05		**N.S**		
Interaction B x C				
		C0	**C1**	**C2**
B0		25.90	27.80	25.30
B1		37.30	40.90	37.80
LSD 0.05		**N.S**		
Interaction A x B x C				
		C0	**C1**	**C2**
A0	**B0**	14.60	13.30	21.10
	B1	30.20	34.60	35.00
A1	**B0**	28.80	42.90	21.10
	B1	33.90	42.30	33.30
A2	**B0**	34.20	27.20	33.60
	B1	47.60	45.80	45.20
LSD 0.05		**N.S**		

Table (6): Effect of vermicompost, seaweed extracts and nitrogen fertilizer on V_{max} of L-Asparatase enzyme (µg N-NH_4^+ g^{-1} soil $24h^{-1}$) at flowering stage

Vermicompost levels (ton ha^{-1})	**A0**	**A1**	**A2**
	135.0	280.0	409.0
LSD .05	**57.4**		
Seaweed extracts (Kg ha^{-1})	**B0**	**B1**	
	198.0	352.0	
LSD .05	**46.8**		
Nitrogen levels (Kg N ha^{-1})	**C0**	**C1**	**C2**
	268.0	288.0	269.0
LSD .05	**N.S**		
Interaction A x B			
	B0	**B1**	
A0	62.0	209.0	
A1	216.0	345.0	
A2	316.0	502.0	
LSD 0.05	**N.S**		
Interaction A x C			
	C0	**C1**	**C2**
A0	114.0	125.0	167.0
A1	265.0	337.0	239.0
A2	424.0	401.0	402.0
LSD 0.05	**N.S**		
Interaction B x C			
	C0	**C1**	**C2**

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B0		198.0	214.0	181.0
B1		337.0	362.0	357.0
LSD 0.05		**N.S**		
Interaction A x B x C				
		C0	**C1**	**C2**
A0	**B0**	52	44	88.0
	B1	177.0	207.0	245.0
A1	**B0**	211.0	284.0	152.0
	B1	319.0	390.0	326.0
A2	**B0**	332.0	314.0	303.0
	B1	517.0	489.0	501.0
LSD 0.05		**N.S**		

Table (7): Effect of vermicompost, seaweed extracts and nitrogen fertilizer on K_m of L-Asparatase enzyme activity (mM) at maturity stage

Vermicompost levels (ton ha^{-1})		**A0**	**A1**	**A2**
		7.55	12.57	10.63
LSD .05		**2.50**		
Seaweed extracts (Kg ha^{-1})		**B0**		**B1**
		10.45		10.05
LSD .05		**N.S**		
Nitrogen levels (Kg N ha^{-1})		**C0**	**C1**	**C2**
		8.91	10.70	11.14
LSD .05		**N.S**		
Interaction A x B				
		B0		**B1**
A0		6.02		9.08
A1		14.76		10.38
A2		10.57		10.68
LSD 0.05		**3.54**		
Interaction A x C				
		C0	**C1**	**C2**
A0		10.08	4.51	8.07
A1		11.54	16.03	10.13
A2		5.11	11.56	15.21
LSD 0.05		**4.33**		
Interaction B x C				
		C0	**C1**	**C2**
B0		12.72	8.62	10.02
B1		5.10	12.79	12.25
LSD 0.05		**3.54**		
Interaction A x B x C				
		C0	**C1**	**C2**
A0	**B0**	10.98	0.82	6.27
	B1	9.18	8.20	9.88
A1	**B0**	18.53	17.06	8.69
	B1	4.56	15.01	11.57
A2	**B0**	8.64	7.98	15.09
	B1	1.58	15.15	15.32

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LSD 0.05	**N.S**

Table (6): Effect of vermicompost, seaweed extracts and nitrogen fertilizer on V_{max} of L-Asparatase enzyme (µg $N\text{-}NH_4^+$ g^{-1} soil $24h^{-1}$) at maturity stage

Vermicompost levels (ton ha^{-1})		**A0**	**A1**	**A2**
		25.7	44.8	30.0
LSD .05		6.8		
Seaweed extracts (Kg ha^{-1})		**B0**	**B1**	
		33.3	39.0	
LSD .05		5.6		
Nitrogen levels (Kg N ha^{-1})		**C0**	**C1**	**C2**
		35.1	36.8	36.6
LSD .05		N.S		
Interaction A x B				
		B0	**B1**	
A0		20.1	31.2	
A1		37.4	52.1	
A2		42.4	33.6	
LSD 0.05		9.7		
Interaction A x C				
		C0	**C1**	**C2**
A0		36.3	16.9	23.8
A1		42.6	57.4	34.4
A2		26.3	36.1	51.7
LSD 0.05		11.9		
Interaction B x C				
		C0	**C1**	**C2**
B0		38.5	27.9	33.6
B1		31.6	45.7	39.7
LSD 0.05		9.7		
Interaction A x B x C				
		C0	**C1**	**C2**
A0	**B0**	39.7	5.1	15.6
	B1	33.0	28.7	31.9
A1	**B0**	13.6	55.0	25.7
	B1	53.6	59.7	43.1
A2	**B0**	44.3	23.5	59.4
	B1	8.2	48.6	44.0
LSD 0.05		**16.8**		

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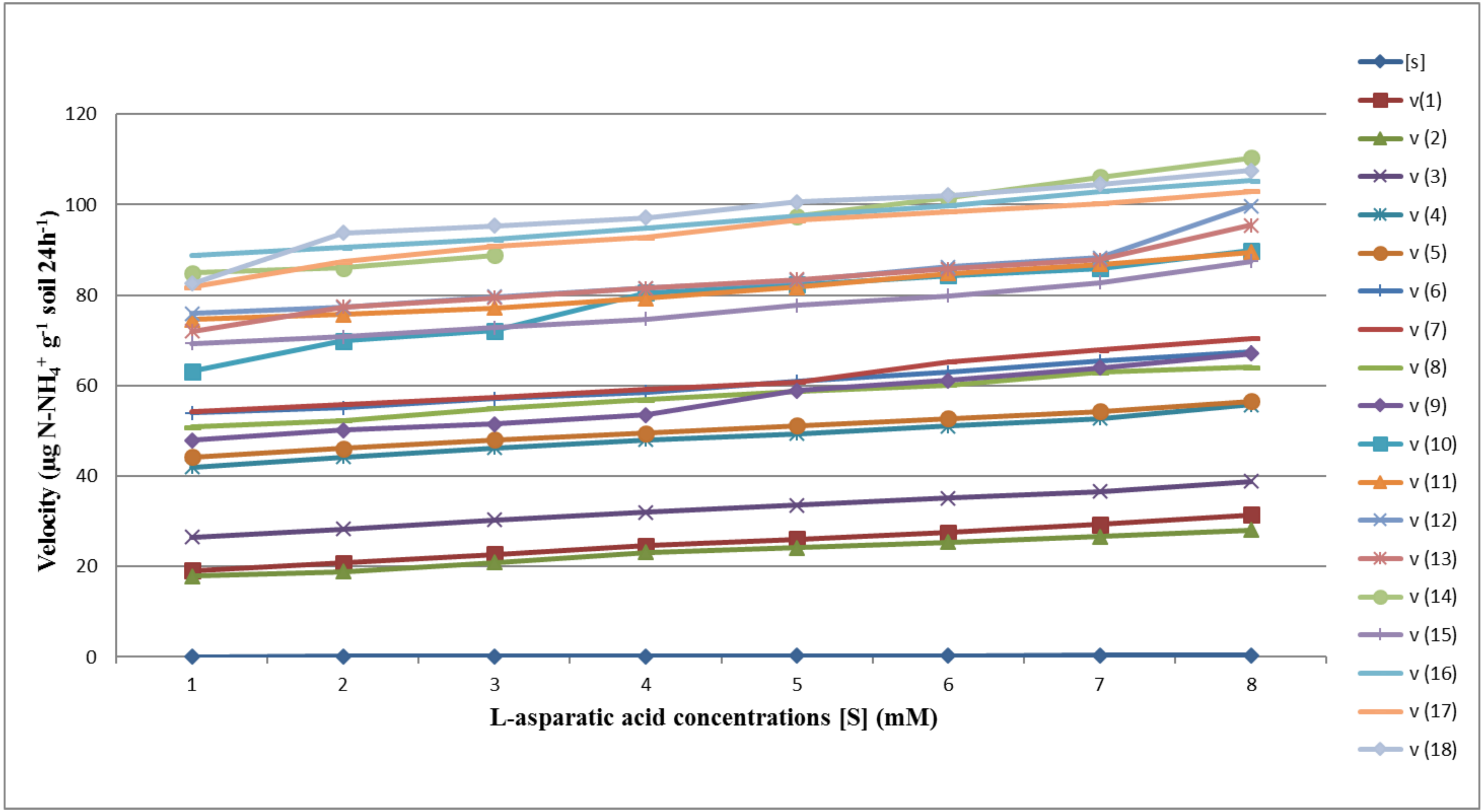

Fig (1): Relationship between L-asparatic acid concentrations [S] and its degradation velocity (V) at flowering stage

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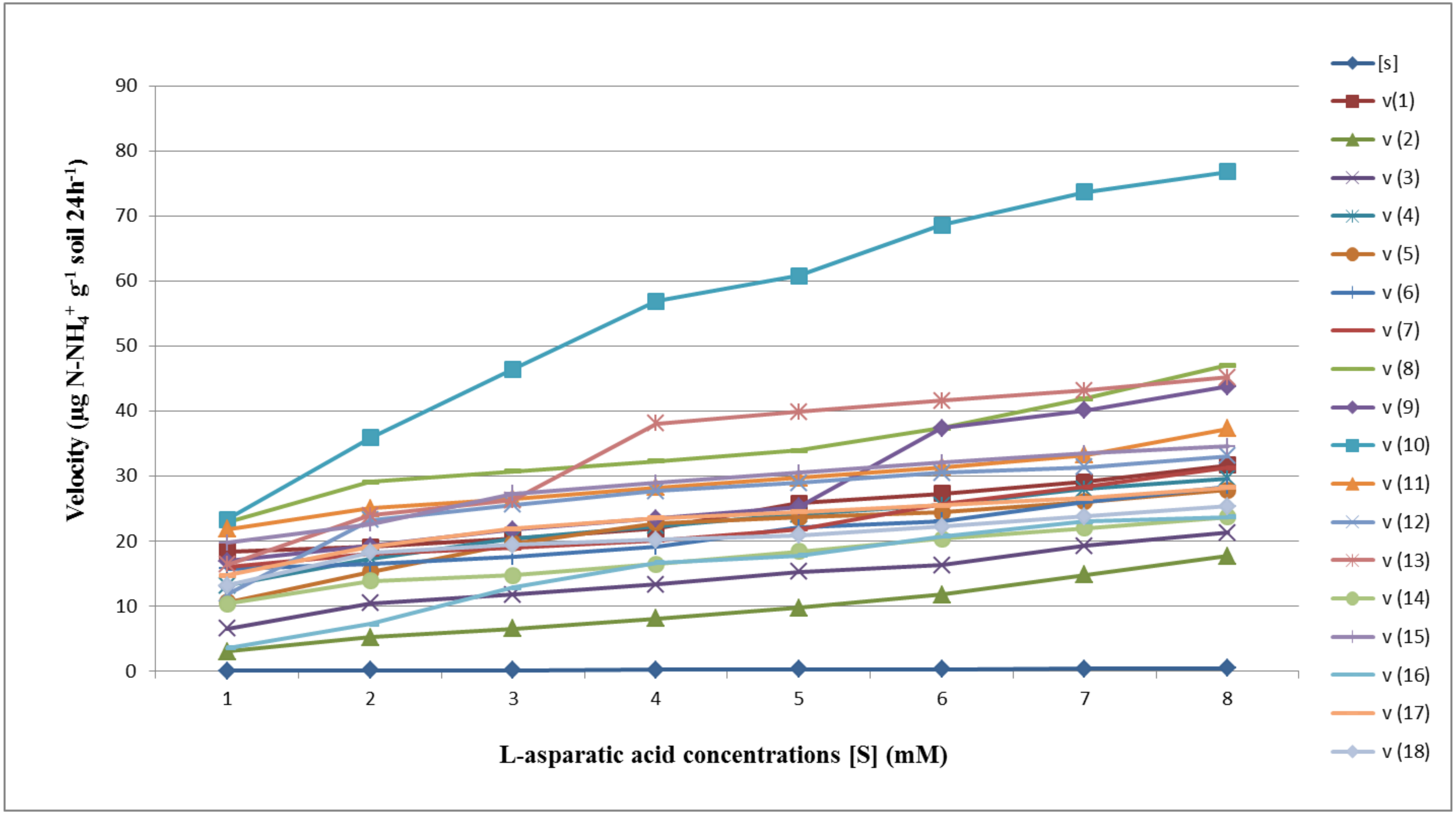

Fig (2): Relationship between L-asparatic acid concentrations [S] and its degradation velocity (V) at maturity stage

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