

Original Research Article

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Effect of Irrigation Schedule and Amino Acids Biostimulants on Soil Enzyme Activities in Potato (*Solanum tuberosum* L.) Crop

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ABSTRACT

Keywords

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The field experiment was conducted alluvial soil of Indo-Gangatic plain at the Institute of Agricultural Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India. The experiment of potato crop was laid out in a split-plot design with three irrigation schedules (I₁, I₂ and I₃) and four amino acid based biostimulants (A₀, A₁, A₂ and A₃) and after harvested soil sample analysis soil enzyme activities. The effect of irrigation on enzyme activities dehydrogenase, protease and alkaline phosphatase was observed non-significant and effect of amino acid based biostimulants was significant. In the interaction effect of irrigation and amino acid based biostimulants on enzyme activities best performance observed in dehydrogenase activities I₃A₂ in first experiment and second experiment I₂A₂. Protease activities was observed highest in I₂A₂ both the experiment and alkaline phosphatase activities was observed highest in I₂A₂ in first experiment and I₁A₂ in second experiment. All the interaction effect on enzyme activities was observed non significantly effect.

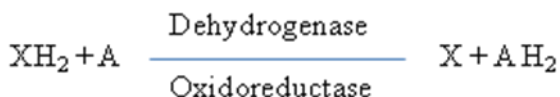
Introduction

Soil is a living system where all the biochemical activities proceed through enzymatic process (Kiss *et al.*, 1975). Soil enzyme activity can be used as an indicator of soil quality for assessing the sustainability of agricultural ecosystems (Roldan *et al.*, 2005). Soil enzymes play an essential role in the nutrient mineralization and their activity is an exceptional 'sensor' in predicting the capacity of nutrient supply to plants. They may correlate with nutrient availability (Asmar *et al.*, 1994) in soil. Bacteria and fungi

synthesize and secrete enzymes such as phosphatases, proteases and pectinases extracellular. Those microbial secreted enzymes constitute an important part of the soil matrix as extra-cellular enzymes, also called abiotic enzymes (Sinsabaugh, 1994). Factors influencing soil microbial activity exert control over soil enzyme production and control on nutrient availability and soil fertility (Sinsabaugh *et al.*, 1994). In particular, phosphatase production and activity have been reported to be very sensitive to soil organic matter concentration (Goldstein *et al.*, 1988). Phosphatases are involved in the

transformation of organic and inorganic phosphorus compounds in soil (Amador *et al.*, 1997) and their activities are an important factor in maintaining and controlling the rate of P cycling through soils. These released phosphorus in soil improved the P content in potato by enhancing P uptake by crop. On the other hand, the increase of protease activity by the application of amino acid biostimulant application may have positive effects on the activity of the others enzymes involving N-cycles since it is one of the enzymes that break down labile polypeptide and other protein polymers. Thus, released N in the form NH_4^+ to NO_3^- enhanced the N-content and protein content in potato.

Biological oxidation of organic compounds is generally a dehydrogenation process and there are many dehydrogenases (enzyme catalyzing dehydrogenation), which are highly specific.

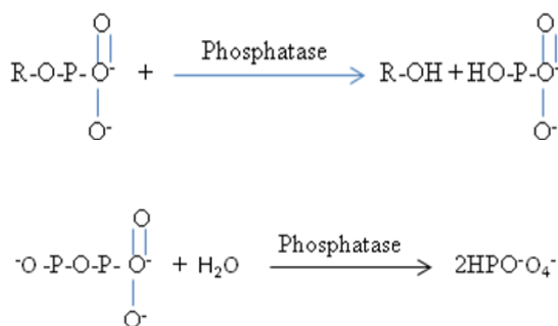


Where, XH_2 is an organic compound (hydrogen donor) and A is a hydrogen acceptor. The dehydrogenase enzyme systems apparently fulfill a significant role in oxidation of soil organic matter as they transfer H from subtracts to acceptors (Skujins, 1967).

Thus dehydrogenase enzyme activity enhancement in soil led to degradation of soil organic matter and release of N, P and S. The released S in soil enhanced the uptake of S by crop and content in tubers. Protein in plant and animal residues are hydrolyzed by protease enzyme. Enzyme protease attacks the protein molecule and hydrolyzes the peptide ($-\text{CO}-\text{NH}$) bonds and release free amino acids *viz.*, peptones and peptides.



Proteases play an important role in mineralization of soil nitrogen. They are involved in cleavage of proteins to polypeptides and oligopeptides to amino acids. The phosphatases are divided in to five group *viz.*, phosphoric monoester hydrolases, phosphoric diester hydrolases, triphosphoric monoster hydrolases, anhydride acting enzymes, P-N phosphoamidases. General equation of the hydrolysis of organophosphorus compounds in soil. The phosphatase enzymes are classified as acid and alkaline phosphatases.



Amino acid N contents in soils (Chen *et al.*, 2002) between 30-45% of the total N. It occurs mostly in soils as single compounds, peptides and proteins bound to clay minerals external and internal surfaces and to humic colloids by hydrogen and covalent bonds. Small amounts of free amino acids occur in the soil liquid phase.

They may originate during the conversion of protein N to NH_3 by heterotrophic organisms, as excretory products of invertebrates, and secreted by microorganisms and plant roots. In general, the amino acid composition of soils shows great similarities to that of bacteria, thus indicating that most of the amino acids present (Schnitzer, 1991) in soil are of microbial origin. The activities of enzymes involved in the different nutrient cycles *viz.* dehydrogenase (S-cycle) protease (N-cycle) phosphatase (P-cycle) were estimated after harvesting the field experiment of potato crop

soil in different treatment combination of irrigation levels (I₁, I₂ and I₃) and sources of amino acid (A₁, A₂ and A₃) spraying during field experiment of potato crop.

Materials and Methods

Experiment field site description

The field experiment was conducted for two consecutive years (2014-15 and 2015-16) which is research farm situated at North – East plains Zone of the Eastern part of Uttar Pradesh at 25° 18' North Latitude 83° 36' East longitudes and at an altitude of 80.71 meters above mean sea level in the Gangatic plain of eastern Uttar Pradesh, India. The district Varanasi rainfalls in a semi-arid to sub-humid climate and means annual precipitation is 1100 mm.

The area occasionally experiences winter cyclonic rain during December to February. In term of percentage of total rainfall, about 84% is received from June to September, 0.7% October to December, 6% from January to February and 9.3 % from March to May as pre monsoon rain (Fig. 1).

The rainfall, relative humidity and wind speed high in first year cultivation period and temperature, sunshine and evaporation high in second year cultivation period.

Experimental conditions

The trial was laid out in a split-plot design with three irrigation schedules (I₁, I₂ and I₃) in main-plots and four amino acid based biostimulants (A₀, A₁, A₂ and A₃) in sub-plots with three replications. The variety of potato (E-3797) tuber seeds was planted *rabi* season in the month of November at a spacing of 50 cm ridge to ridge and 20 cm plant to plant. The recommended dose of fertilizers for the crop was 150:100:120 NPK kg ha⁻¹ at basal

application of ½ doses N + full dose of phosphorus + full dose of potash. The main plot treatment of irrigation schedule water applied at I₁ (3 irrigation), I₂ (4 irrigation) and I₃ (5 irrigation). The sub-plot applied four types (A₀, A₁, A₂ and A₃) of amino acid based biostimulants of (A₀) control, (A₁) plant based, (A₂) animal based and (A₃) mixture amino acid at three application schedule of amino acid. The first application of amino acid based biostimulants at five leaves stage of the crop, second application at tuber initiation stage and third application at fifteen days after second stage of application. All the biostimulants are in liquid formulation (250 mL of amino acid/100L of water, ha⁻¹) and will be applied through sprayer, mixing/diluting with water.

Collection of soil sample

Soil samples collected from field experiment of potato crop at after harvesting, stored at 4⁰C for analysis of assay enzyme activities. For the impact assessment of amino acid biostimulants under different irrigation schedule on soil enzyme activities, viz., dehydrogenase, protease and alkaline phosphatase were analyzed.

Enzyme activities measurements

Dehydrogenase

Dehydrogenase activity in the soil sample was estimated by (Casida *et al.*, 1964) method.

Protease

Protease activity was determined by (Reysek *et al.*, 2008) methods.

Alkaline phosphatase

Activity of alkaline phosphatase estimation in soil was described (Tabatabai and Bremner, 1969).

Enzyme activities cycle in soil

Water availability plays a role in the performance of soil microbial communities in natural (Fig. 2) and agricultural ecosystems at the level of microbial growth, biomass (Fierer *et al.*, 2003; Hueso *et al.*, 2011; Hueso *et al.*, 2012; Meisner *et al.*, 2013) microbial composition and biogeochemical cycles (Placella *et al.*, 2012; Goransson *et al.*, 2012).

The low irrigation dose, increases the alkaline phosphatase activity were observed in the soils and at high irrigation, dehydrogenase activity was significantly decreased in commercial fertilized soil, relative to non-fertilized soil and pine bark, ash, and N (Moreno *et al.*, 2017).

Results and Discussion

The enzyme activities in field experiment effect of irrigation on dehydrogenase activities was found highest in 4 irrigation schedule and decrease after 3 irrigation, 5 irrigation schedule respectively in first year experiment (Table 1) and the second year field experiment soil effect of irrigation on dehydrogenase activities was found highest activities in 3 irrigation schedule, 4 irrigation and 5 irrigation schedule respectively. The entire irrigation schedule both the year was found non-significant effect of dehydrogenase activities.

The enzyme activities of protease effect of irrigation was found highest in 3 irrigation schedule both the year of experiment and after decrease 4 irrigation, 5 irrigation schedule respectively both the year of experiment (Table 1). The activities of protease were found all the effect of irrigation schedule was non-significantly effect. The enzyme activities of dehydrogenase and protease was found second year highest than first year experiment

and the time of cultivation period rain fall was found highest (0.99 mm) than second year rain fall (0.30 mm) in cultivation period. The effect of higher irrigation schedule decreases the activities of dehydrogenase and protease activates. The activity of alkaline phosphatase effect of irrigation was found highest in 4 irrigation schedule and after 5 irrigation and 3 irrigation schedules respectively (Table 1), in first year experiment soil and in second year activities highest in 3 irrigation schedule after 5 irrigation and 4 irrigation schedules respectively. All the effect of irrigation schedule was found non significantly effect of alkaline phosphatase in soil.

The soil enzyme activities, viz., dehydrogenase, protease and alkaline phosphatase were significantly increased in the harvested soil due to application of different sources of amino acids in potato crop.

Maximum activity (Fig. 3) of dehydrogenase (689.97 and 912.67 $\mu\text{g g}^{-1}$ TPF day^{-1} soil), protease (8.31 13.38 $\mu\text{g L}^{-1}$ tyrosine g^{-1} soil h^{-1}) (Fig. 4) and alkaline phosphatase (Fig. 5) (43.19 and 33.30 $\mu\text{g PNPg}^{-1}$ soil h^{-1}) in harvested soil were observed due to application of plant based amino acids (A_2) in both the year.

The enzyme activities of dehydrogenase and protease was found highest in second year than first year experiment at the time of cultivation period rain fall was observed highest (0.99 mm) than second year rain fall (0.30 mm) in cultivation period. Thus, overall the order of impact on enzyme activities in soil due to application of amino acids biostimulants was $A_2 > A_1 > A_3$. These results are same with many others reported in the literature, showing enhancement soil enzyme activities are correlated with biostimulant application (Chen *et al.*, 2002; Chen, 2003).

Fig.1 Meteorological data of Varanasi district during the cultivation period (November to March) of potato crop

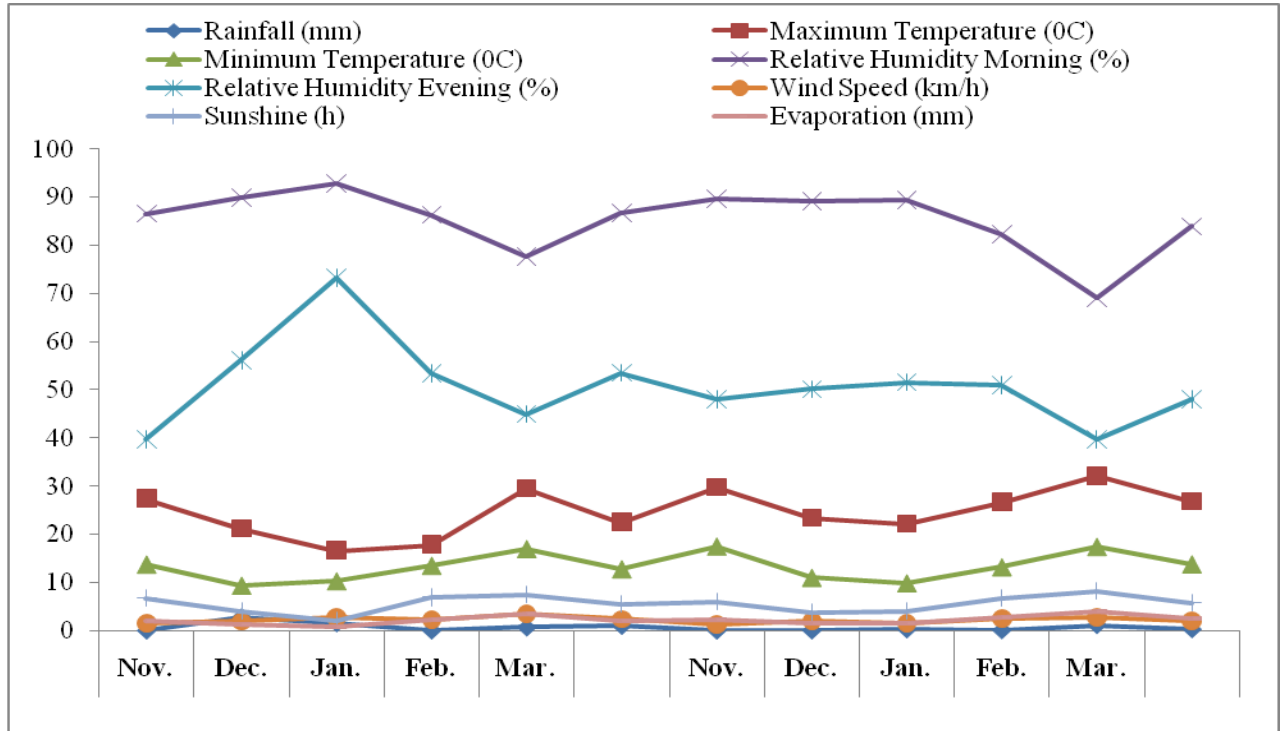


Fig.2 Effect of irrigation and amino acids based biostimulants on enzyme activities cycle in soil

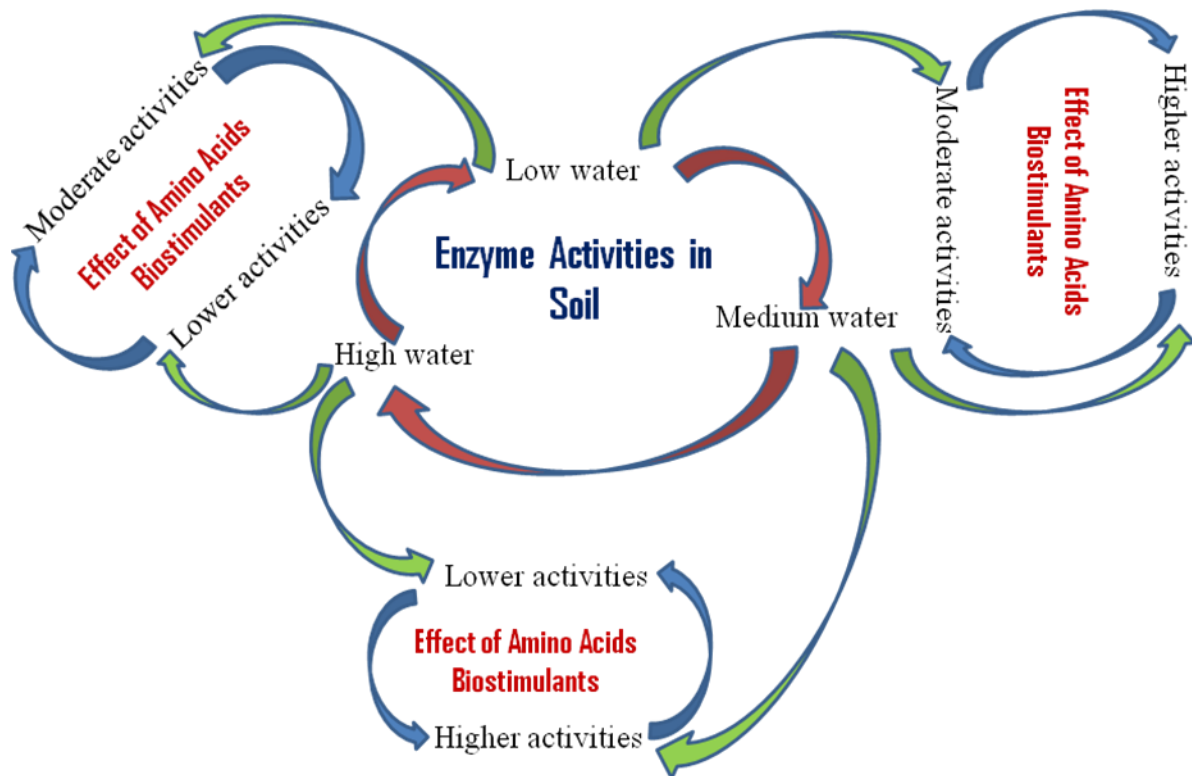


Fig.3 Effect of amino acids based biostimulants on dehydrogenase

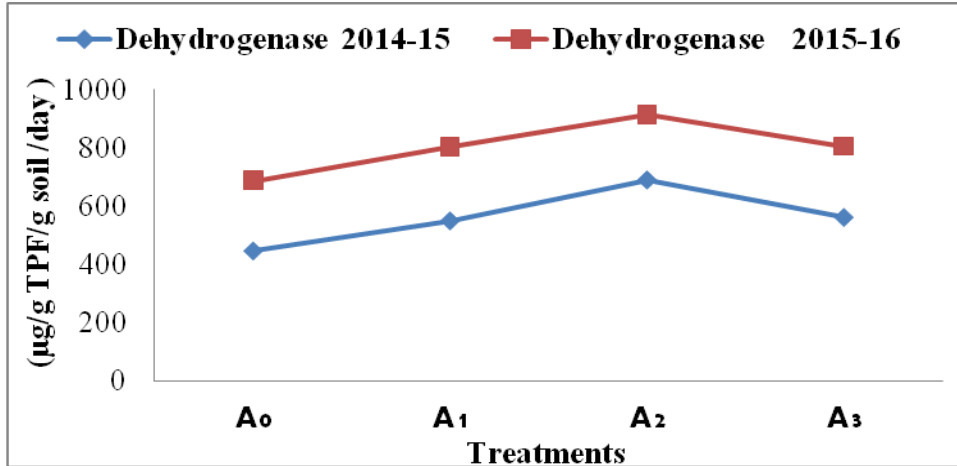


Fig.4 Effect of amino acids based biostimulants on protease activities

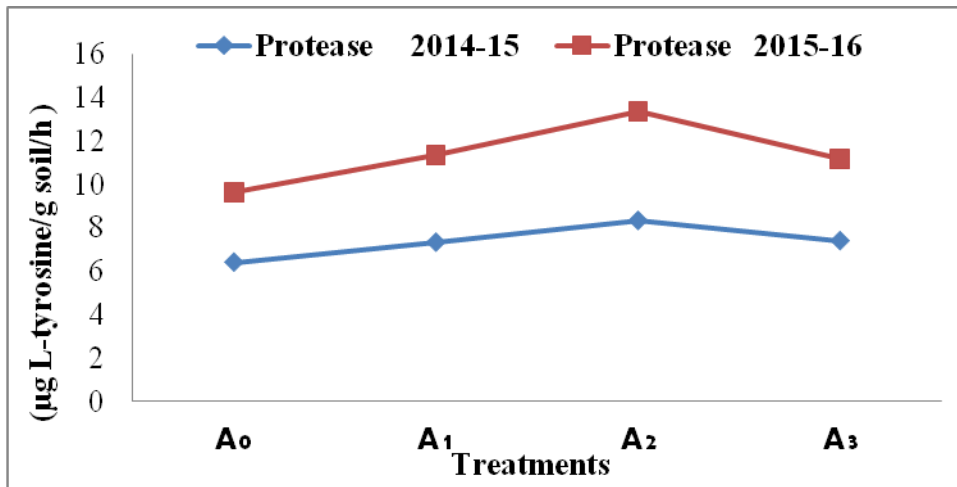


Fig.5 Effect of amino acids based biostimulants on alkaline phosphatase activities

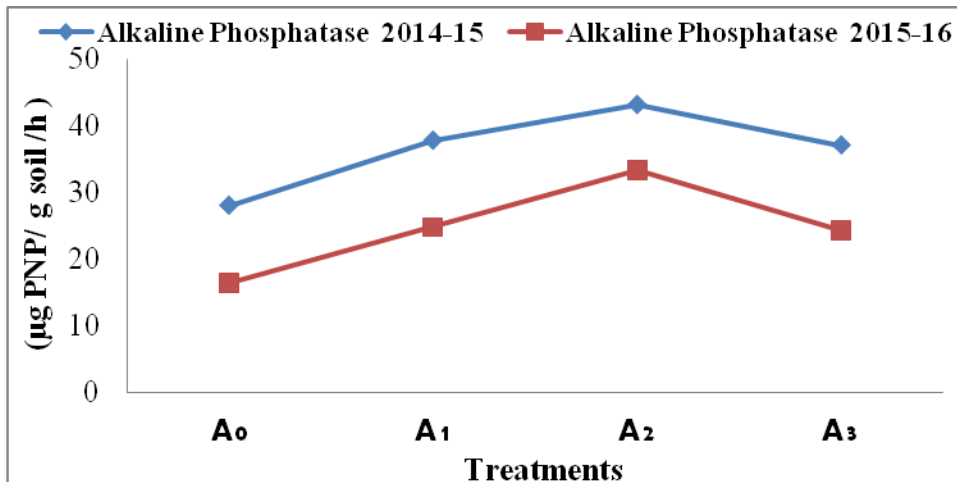


Table.1 Effect of irrigation on enzyme activities

Treatments	Dehydrogenase ($\mu\text{g g}^{-1}$ TPF g^{-1} soil day^{-1})		Protease ($\mu\text{g L-tyrosine g}^{-1}$ soil h^{-1})		Alkaline Phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1})	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
I ₁	551.02	836.72	7.41	11.79	34.44	28.13
I ₂	587.56	794.77	7.37	11.37	38.21	22.67
I ₃	546.66	771.62	7.26	11.03	36.95	23.24
SEm (\pm)	NS	NS	NS	NS	NS	NS
CD (P=0.05)	NS	NS	NS	NS	NS	NS

Table.2 Interaction effect of irrigation and amino acids based biostimulants on enzyme activities of soil at the time of harvesting of experiment field

Treatments	Dehydrogenase ($\mu\text{g g}^{-1}$ TPF g^{-1} soil day^{-1})		Protease ($\mu\text{g L-tyrosine g}^{-1}$ soil h^{-1})		Alkaline Phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1})	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
I ₁ A ₀	469.74	726.21	6.98	10.13	26.91	18.11
I ₁ A ₁	522.25	835.31	7.55	11.82	37.09	27.28
I ₁ A ₂	672.59	910.84	7.6	13.64	42.99	42.11
I ₁ A ₃	539.49	874.52	7.51	11.58	30.76	25.03
I ₂ A ₀	491.79	684.31	6.17	9.08	29.52	17.92
I ₂ A ₁	572.13	780.95	7.23	11.93	38.7	23.82
I ₂ A ₂	678.94	941.79	9.03	13.91	44.74	24.19
I ₂ A ₃	607.37	772.03	7.03	10.55	39.89	24.75
I ₃ A ₀	377.01	648.08	6.01	9.70	27.61	13.21
I ₃ A ₁	553.99	788.95	7.14	10.36	37.67	23.25
I ₃ A ₂	718.38	885.07	8.28	12.59	41.84	33.60
I ₃ A ₃	537.27	764.37	7.6	11.48	40.68	22.90
SEm \pm	NS	NS	NS	NS	NS	NS
CD = 0.05)	NS	NS	NS	NS	NS	NS

In the interaction effect of irrigation and amino acid based biostimulants on enzyme activity with 5 irrigation schedule and plant based amino acid biostimulants (I_3A_2) was best performance (718.38, $\mu\text{g/g TPF/g soil/day}$) dehydrogenase activities in first year experiment and second year (I_2A_2) 4 irrigation schedule and plant based amino acid biostimulants (941.79 $\mu\text{g/g TPF/g soil/day}$) in (Table 2). The effect of irrigation and amino acid based biostimulants on Protease activities was observed highest in 4 irrigation schedule and plant based amino acid biostimulants (I_2A_2) both the year of experiment and alkaline phosphatase activities was observed highest in (I_2A_2) 4 irrigation schedule and plant based amino acid biostimulants (44.74, $\mu\text{g PNP/g soil/h}$) in first year and (I_1A_2) 3 irrigation schedule and plant based amino acid biostimulants (42.11, $\mu\text{g PNP/g soil/h}$) in second year experiment. All the interaction effect of irrigation and amino acid based biostimulants on enzyme activities dehydrogenase, protease and alkaline phosphatase was observed non-significant effect.

In the cultivation period effect of climatic condition in first year- rainfall (0.99 mm), temperature (maximum 22.52 $^{\circ}\text{C}$ and minimum 12.79 $^{\circ}\text{C}$), relative humidity (morning 86.58% and evening 53.60%), wind speed (2.37 km h^{-1}) sunshine (5.47 h) and evaporation (1.98 mm) and second year climatic condition-rainfall (0.30 mm), temperature (maximum 26.83 $^{\circ}\text{C}$ and minimum 13.80 $^{\circ}\text{C}$), relative humidity (morning 83.83% and evening 48.14%), wind speed (2.01 km h^{-1}) sunshine (5.76 h) and evaporation (2.36 mm) was observed climatic variability on dehydrogenase and protease activities, over all dehydrogenase and protease activities interaction highest in second year experiment than first year experiment and the alkaline phosphatase activities over all interaction highest in first year experiment than second year experiment.

The different levels of irrigation schedules (I_1 , I_2 and I_3) during crop cultivation were non significantly influenced the enzyme activities

(viz. dehydrogenase, protease and alkaline phosphate) in both years of cultivation after harvesting the soil. The soil enzymes related to protein transformation (protease), organic phosphorus transformation (alkaline phosphatase) and organic matter decomposition (dehydrogenase) activities greatly improved by the spraying of plant based (A_2), animal source (A_1) and mixtured amino acids (A_3) on crops. The soil enzyme activities (dehydrogenase, protease and alkaline phosphatase) were significantly enhanced by the spraying of all the amino acid based biostimulants on crop and the order of overall impact of soil enzyme activities in soil due to application of amino acids biostimulants was $A_2 > A_1 > A_3$. Thus, amino acids application improved the soil health plant growth and development.

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