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IMPACT OF INTRODUCED BIOINOCULANTS ON UREASE ENZYME ACTIVITY IN SOIL UNDER DIFFERENT LEVELS OF DRIP IRRIGATION AND FERTIGATION IN SUGARCANE CULTIVATION

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ABSTRACT:

The study entitled "Impact of introduced bio inoculants on urease enzyme activity in soil under different levels of drip irrigation and fertigation in sugarcane cultivation" is done to evaluate the effect of bio inoculants (*Azospirillum*, Phosphobacteria and Potash solubilizers) and different irrigation levels on urease activity and thereby nitrogen recovery by sugarcane plant. In this study sugarcane variety CO 86032 was used with different levels of irrigation (75%, 100%, 125% Recommended dose of irrigation) and fertigation (75% and 100% Recommended dose of fertilizers with different formulations of fertilizers and with or without bio fertilizers). Bio fertilizers were applied at 30, 60 and 90 DAP. Based on the results obtained from the field trial, Application of 125 percent ETc (I_3) showed maximum urease activity at 150 DAP and 75 percent RDF as fully water-soluble fertilizer + LBF (F_5) recorded the highest urease activity and the Interaction of 125 percent irrigation with 100 percent RDF as fully water-soluble fertilizer (I_3F_6) and 100 percent irrigation with 75 percent RDF as fully water-soluble fertilizer + LBF (I_2F_5) showed the highest urease activity.

KEYWORDS: *Urease ; Bio fertilizer; Soil Enzymes; Sugarcane.*

INTRODUCTION:

Sugarcane (*Saccharum officinarum)* is a tropical plant that requires more

water and nutrients. India is one of the major producers of sugar and sugarcane. Sugarcane responds well to higher N application rates. Climate and biotic factors are the major key driver of crop growth (Antony *et al*., 2020), N demand and N loss processes. Although sugarcane requires large inputs of N for successful crop growth, it is relatively inefficient in the recovery of N fertilizer. Recovery studies of applied N fertilizer in the crop and surrounding soil indicate maximum recoveries are just over 60 % of N applied. The unrecovered N is either held in the soil by microbial immobilization and/or lost from the sugarcane production system. The magnitude of N losses and low recoveries of fertiliser N by the sugarcane crop are of significant economic and environmental importance (Llyod and Jane, 1973).

Nitrogen losses occur when urea is hydrolyzed mainly due to the activity of soil enzyme, Urease. In most arable soil urea is converted into ammonia and CO_2 by soil urease (Dharmakeerthi and Thenabadu., 1996). Ureolytic activity minimizes crop damage during urea fertilization of agricultural soil and solves the problem of fixed nitrogen availability (Mobley & Hausinger, 1989).

Soil microorganisms are important components of the soil ecosystem, leading the nutrient cycle and energy flow, meanwhile, it plays an important role in maintaining the eco-system stability and sustainability (Antony *et al*., 2019). 90% of microorganisms involved in the soil reaction process, could change the soil fertilization. Bio fertilizers are the crucial component of the integrated nutrient management (INM) systems (Jeyabal *et al.,* 1999). Bacteria that can hydrolyse urea are common in soils. Some 17–30 percent of the total bacterial populations, including aerobes, micro-aerophiles and anaerobes, could hydrolyse urea. When available carbon as glucose was added with urea to this soil, urease activity and size of the bacterial population both increased, but the ratio of ureolytic to non-ureolytic bacteria in the population remained unchanged. (Llyod and Jane, 1973)

Soil is a living system in which biological activities take place with the help of enzymatic processes. Quantitative measurement of soil enzyme activities can contribute to our understanding of these biological transformations by allowing us to evaluate the activity present in the soil. This study is taken to analyse the impact of introduced bio fertilizers on the urease activity in the soil to understand the nitrogen nutrition of the sugarcane crop.

MATERIAL AND METHOD:

The experiments were carried out at the central farm of Agricultural College and Research Institute, Madurai with the following treatments in spilt plot design having three main field treatments and six subplot treatments. 75%, 100% 125% Recommended dose of irrigation as main plot treatments (I_1, I_2, I_3) and fertilizer dose in subplot treatment (F_1-75% RDF –

commercial fertilizer + liquid bio fertilizer, F_2-100%RDF – Commercial fertilizer, F_3 -75% RDF - 50% commercial fertilizer and 50% water-soluble fertilizer +Liquid bio fertilizer , F_4-100%RDF - 50% commercial fertilizer and 50% water-soluble fertilizer, F_5-75% RDF - Fully water-soluble fertilizer + LBF, F_6-100%RDF - Fully water-soluble fertilizer).

Sugarcane variety CO 86032 was used for the research and the spacing used is 165cm. The recommended fertilizer dose of fertilizer followed is 344:94:169 kg NPK ha^{-1}. Three types of liquid biofertilizers (*Azospirillum*, Phosphobacteria and Potash solubilizers formulated by IPL Ltd) were given through subsurface drip system at 30, 60 and 90th DAP.

Soil samples were collected from the corners and the canter of the field at 0-15 cm depth during the cropping period at 30 days interval, samples were combined and then thoroughly mixed to obtain a homogenous mixture. The soil was taken directly for enzyme assay. The portion of the soil was dried and taken for soil nutrients analyses.

Urease hydrolyzed urea into ammonia and the resulting release of ammonia was measured by Nesselerization. (Jackson, 1973 and Zantua and Bremner, 1975).

Ten grams of soil sample were incubated with 20 ml of THAM buffer (6.1 g of tris (hydroxymethyl) aminomethane in 700 ml of water, brought pH of the solution to 6.5 by addition of 0.2 M sulfuric acid and dilute with water to 1 lit.) at pH 6.5 containing 2% urea and added 0.2 ml toluene. Soil without substrate served as a control. The flasks were tightly stoppered and incubated for 2 h at 37^{o}C. The extra ammonia formed as a hydrolysis product of urea over control which did not receive any urea solution was extracted with Ag_2SO_4- KCl solution (100 mg Ag_2SO_4 in 700ml of water, dissolved 188 g of reagent-grade KCl in the solution and dilute to 1 lit.). The ammonia content in aqueous extract was measured by Nesslerization. One ml of the extract was taken in a 100 ml standard flask, in which 2 ml of 10% sodium potassium tartrate solution, 2ml of 10% acidified sodium chloride (10g NaCl dissolved in 100 ml of ammonia-free distilled water and the pH was adjusted to 2.5 with dilute hydrochloric acid) and 5 ml of Nessler's reagent were added. The tubes were allowed to stand for 30 min and then the absorbance was measured at 410 nm in a Bausch and lamb spectronic 20 colorimeter. The enzyme activity was expressed as mg NH_4^+ formed /g of soil per hour.

RESULTS AND DISCUSSION:

The urease activity was recorded at constant intervals and it was significantly influenced by the irrigation and fertigation levels. The results are presented in table 1 and fig 1.

The urease activity was significantly increased in 125 percent irrigation (I_3). It recorded the maximum urease activity (50.0 µg NH_3 g^{-1} hr^{-1}) at 150 DAP. The lowest was recorded in 75 percent irrigation (I_1) (47.0 µg NH_3 g^{-1} hr^{-1}).

Among the fertigation levels, 75 percent RDF as fully water-soluble fertilizer + LBF (F_5) (60.3 µg NH_3 g^{-1} hr^{-1}) at 150DAP showed the highest urease activity. The lowest activity was recorded in 100 percent RDF as fully commercial fertilizer (F_2) (21 µg NH_3 g^{-1} hr^{-1}).

Interaction effect of 125 percent irrigation with 100 percent RDF as fully water-soluble fertilizer (I_3F_6) and 100 percent irrigation with 75 percent RDF as fully water-soluble fertilizer + LBF (I_2F_5) showed the highest urease activity (61 µg NH_3 g^{-1} hr^{-1}). The lowest urease activity occurred in 75 percent irrigation with 100 percent RDF as fully commercial fertilizer (I_1F_2) (37.0 µg NH_3 g^{-1} hr^{-1}) equal to 75 percent irrigation with 100 percent RDF as fully water-soluble fertilizer (I_1F_6).

The urease activity in the present study was increasing up to 150 DAP. Then it started to decline till the harvest of the crop. The maximum urease activity was observed in the treatment of 125 percent Irrigation with 75 percent RDF as fully water-soluble fertilizer + LBF (I_3F_5). This may due to the higher moisture content along with the introduced biofertilizer with optimum nutrient content. The number of microbes present in that particular treatment might induce urease activity. Urease activity in soil appears to be correlated with the number of microbes and it is more in the rhizosphere (Rice,1971). Organic matter content also one of the reasons for the higher urease activity. Koepf (1954) found that the urease activity varied with soil type and increased with increasing organic matter. Galstyan (1958) noted that organic and mineral fertilizers increased urease activity. In this study Urease activity might be influenced by potassium application (Saravanan, 1996). Soil urease activity was positively correlated with increasing total nitrogen and phosphorus (Barush and Mishra, 1984).

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Table 1. Impact of Introduced Bioinoculants on Urease Enzyme Activity in Soil Under Different Levels of Drip Irrigation and Fertigation in Sugarcane Cultivation

Treatment	30 DAP				60 DAP				90 DAP				120 DAP			
	I_1	I_2	I_3	MEAN	I_1	I_2	I_3	MEAN	I_1	I_2	I_3	MEAN	I_1	I_2	I_3	MEAN
F_1	20.0	28.0	29.0	25.6	32.0	33.0	36.0	33.6	38.0	41.0	49.0	42.6	52.0	47.0	53.0	50.6
F_2	18.0	22.0	23.0	21.0	22.0	25.0	29.0	25.3	26.0	38.0	33.0	32.3	32.0	40.0	35.0	35.6
F_3	23.0	25.0	31.0	26.3	27.0	37.0	35.0	33.0	32.0	45.0	39.0	38.6	48.0	51.0	51.0	50.0
F_4	20.0	19.0	20.0	19.6	25.0	27.0	25.0	25.6	30.0	27.0	31.0	29.3	34.0	34.0	33.0	33.6
F_5	26.0	27.0	33.0	28.6	36.0	39.0	41.0	38.6	42.0	43.0	47.0	44.0	49.0	55.0	59.0	54.3
F_6	21.0	23.0	24.0	22.6	26.0	26.0	27.0	26.3	31.0	31.0	27.0	29.6	31.0	35.0	39.0	35.0
MEAN	21.3	24.0	26.6		28.0	31.1	32.1		33.167	37.5	37.6		41.0	43.6	45.0	
	I	F	I x F		I	F	I x F		I	F	I x F		I	F	I x F	
SED	0.22	0.18	0.36		0.18	0.27	0.59		0.21	0.35	0.59		0.17	0.46	0.74	
CD(0.05)	0.60	0.37	0.65		0.58	0.71	1.26		0.58	0.71	1.26		0.46	0.94	1.54	

Treatments	150 DAP				180 DAP				210 DAP				AT HARVEST			
	I_1	I_2	I_3	MEAN	I_1	I_2	I_3	MEAN	I_1	I_2	I_3	MEAN	I_1	I_2	I_3	MEAN
F_1	56.0	51.0	58.0	55.0	48.0	44.0	49.0	47.0	43.0	39.0	42.0	41.3	38.7	35.1	37.8	37.2
F_2	38.0	43.0	41.0	40.6	33.0	38.0	35.0	35.3	29.0	36.0	30.0	31.6	26.1	32.4	27.0	28.5
F_3	55.0	58.0	56.0	56.3	45.0	51.0	47.0	47.6	40.0	43.0	43.0	42.0	36.0	38.7	38.7	37.8
F_4	37.0	39.0	39.0	38.3	31.0	36.0	37.0	34.6	28.0	28.0	34.0	30.0	25.2	25.2	30.6	27.0
F_5	59.0	61.0	61.0	60.3	53.0	58.0	49.0	53.3	41.0	54.0	44.0	46.3	36.9	48.6	39.6	41.7
F_6	37.0	40.0	45.0	40.6	31.0	33.0	40.0	34.6	28.0	31.0	38.0	32.3	25.2	27.9	34.2	29.1
MEAN	47.0	48.6	50.0	48.5	40.1	43.3	42.8	42.1	34.8	38.5	38.5	37.2	31.3	34.6	34.6	33.5
	I	F	I x F		I	F	I x F		I	F	I x F		I	F	I x F	
SED	0.12	0.47	0.75		0.14	0.41	0.59		0.17	0.35	0.59		0.16	0.32	0.53	
CD(0.05)	0.34	0.95	1.65		0.48	0.72	1.23		0.48	0.72	1.23		0.43	0.65	1.11