D-5849
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RESEARCH ARTICLE Agricultural Science Digest

Effect of Different Levels of Sodicity on Soil Enzymes, Soil Microbial Biomass Carbon and Activity of Antioxidant Enzymes in Different Rice Varieties under Semi-arid Conditions

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10.18805/ag.D-5849

ABSTRACT

Background: Soil sodicity is a major abiotic stress for crop production in many parts of the world. Soil enzymes have been proposed as potential indicators of soil quality due to their connection to soil biology, ease of measurement and quick responsiveness to changes in soil management. Concerning that how different levels of sodicity affects the activity of soil enzymes, soil MBC and antioxidant enzymes in different rice varieties will promote us to understand the mechanism behind the tolerant varieties and to find out the ways to improve the tolerance mechanism.

Methods: A field experiment was conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, Tamil Nadu during September 2022 to January 2023. Different rice varieties *viz.,* TRY 1, CO 43, TRY 2, CSR 27, TRY 3 and white ponni (WP) were grown at different levels of Exchangeable Sodium Percentage (ESP) *i.e.,* 8, 16, 24, 32, 40 and 48 under field condition. The soil samples were collected at flowering stage and analyzed for urease, alkaline phosphatase (APH), dehydrogenase (DHG) and soil microbial biomass carbon (MBC). The plant samples collected at flowering stage were examined for activity of antioxidant enzymes like catalase (CAT) and peroxidase (POX).

Result: The growth and yield decreases with increasing sodicity levels. The soil enzyme and microbial biomass carbon (MBC) found to be decreased with increasing ESP levels. The antioxidant enzyme increases with increase in sodicity levels in tolerant varieties and decreases at high ESP levels in susceptible varieties. Overall TRY 3 outperformed under increasing sodicity levels compared to other varieties.

Key words: Antioxidant, Enzyme activity, Microbial biomass carbon, Rice, Sodicity.

INTRODUCTION

The majority of salt-affected soils are found in arid and semiarid regions. Increased salts accumulate in soils in these regions because evapotranspiration outpaces annual precipitation (Zhao *et al.,* 2020). The biological, physical, and chemical characteristics of soils are severely affected by soils high salt concentrations (Ashrafuzzaman *et al.,* 2022). Increased sodium-based carbonates and bicarbonates significantly deteriorate the physical characteristics of sodic soils (Ayers and Westcot, 1985; Minhas *et al.,* 2007). For the exploitation and utilization of saline-alkaline soils, a comprehensive understanding of the variance in soil enzyme activity is required (Shi *et al.,* 2019). The soil MBC makes up 1% to 3% of the soil total organic carbon, but it also has a high turnover rate and functions as a labile store for nutrients (Marumoto, 1984). The higher levels of sodium-based carbonates and bicarbonates in sodic soil and soil that has been irrigated with sodic water significantly worsen soil properties to raising pH, EC and exchangeable sodium percentage (ESP) (Minhas *et al.,* 2007). As a result, it is anticipated that soil microbial populations and enzyme activities will behave differently in sodic environment.

Catalase (CAT) can prevent damaging effects of sodium by facilitating the peroxide generated during metabolism in plants (Guangming *et al.,* 2017). Nannippori *et al.* (2011) stated that phosphatases play an essential role in converting organic P into inorganic forms that are available to plants. In order to guard against oxidative stress, plant cells produce antioxidant enzymes such peroxidase and catalase (Del Rio *et al.,* 2003) that break down H_2O_2 into water (Gara *et al.,* 2003). Different researchers have previously reported on

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How to cite this article: Surya, M., Baskar, M., Meena, S., Janaki, D., Geethanjali, S. and Sundar, M. (2023). Effect of Different Levels of Sodicity on Soil Enzymes, Soil Microbial Biomass Carbon and Activity of Antioxidant Enzymes in Different Rice Varieties under Semi- arid Conditions. Agricultural Science Digest. DOI: 10.18805/ag.D-5849.

Submitted: 31-07-2023 **Accepted:** 23-08-2023 **Online:** 05-09-2023

dehydrogenase and urease enzymes in relation to salinity and sodicity (Batra and Manna, 1997; Tripathi *et al.,* 2006). Because dehydrogenases occur intracellularly in all living microbial cells, they are one of the most significant enzymes in the soil environment and are used as an indicator of overall soil microbial activity (Salazar *et al.,* 2011). The specific objectives of the study were 1) To determine the effect of different sodicity levels on soil enzymes *viz.,* urease, alkaline phosphatase and dehydrogenase activity 2) To evaluate the microbial biomass carbon content under different levels of sodicity 3) To assess the activity of plant catalase and peroxidase under increasing ESP levels.

MATERIALS AND METHODS

During September 2022 to January 2023 a field experiment was conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli. The experiment was conducted in strip plot design with two replications at six different ESP levels *ie.,* 8, 16, 24, 32, 40 and 48 with six rice varieties V_1, V_2, V_3, V_4, V_5 and V_6 *ie.,* TRY 1 (Trichy 1), CO 43 (Coimbatore 43), TRY 2 (Trichy 2), CSR 27 (Central Salinity research 27), TRY 3 (Trichy 3) and WP (White Ponni). The initial soil properties and ESP maintained were presented in Table 1. The ESP levels were maintained upto critical stage of rice (flowering stage). The soil samples were collected at flowering stage and analyzed for urease, Alkaline phosphatase (APH), Dehydrogenase (DHG) and soil Microbial Biomass Carbon (MBC). The plant samples collected at flowering stage were examined for activity of antioxidant enzymes *viz.,* Catalase (CAT) and Peroxidase (POX).

Urease, alkaline phosphatase and dehydrogenase activities

The non-buffer method of Zantua and Bremner (1975) was used to measure the urease activity. Toluene and 9 ml of tris-hydroxymethyl aminomethane (THAM) buffer (0.05 M) at pH 9.0 were added to 5 g of 2 mm sieved soil in a 50 ml volumetric flask. For two hours at 370°C, the samples were incubated with 1 ml of a 0.2 M urea solution. $KCl.Ag_2SO_4$ solution was added, mixed and NH_4^+-N was estimated in a 20 ml volume using the steam distillation method. The method of Tabatabai and Bremner (1969) was used to assess alkaline phosphatases, utilizing modified universal buffer (pH 11.0), p-nitrophenyl phosphate dibasic as a substrate and incubated at 35°C for one hour. Dehydrogenase activity was determined by 2, 3, 5 triphenyl tetrazolium chloride using 1 g of moist soil and expressed as µg of triphenylformazan (TPF) formed per gram of oven dry soil per 24 hours (Casida *et al.,* 1964).

Soil microbial biomass carbon

According to Vance *et al.* (1987), the microbial biomass carbon was measured using the chloroform fumigation-extraction method. A 20 g of fresh soil was collected and it was fumigated using ethanol-free chloroform for 24 hours in a vacuum desiccator. After that, it was filtered and extracted with 0.5 M K_2SO_4. In a 250 ml conical flask, 10 ml of the filtrate was treated with 10 ml of 0.035 N $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4. The substance was digested for 30 minutes on a hot plate at 150-1700°C before being cooled. It was titrated against 0.04 N FAS after the addition of 25 ml of distilled water and 5 ml of phosphoric acid using diphenylamine indicator. All the processes were completed, excluding fumigation, to create a non-fumigated set. The titration value was used to compute the carbon content. By deducting the extracted carbon from samples that had not been fumigated from samples, MBC was calibrated and expressed as µg g^{-1} of soil.

Catalase and peroxidase activity

According to Gossett *et al.* (1994), the activity of the enzymes catalase and peroxidase was measured. Catalase activity was quantified as ìg of H_2O_2 min^{-1} g^{-1} and peroxidase activity as a change in absorbance at 430 nm min^{-1} g^{-1}, respectively. For estimation of catalase activity 0.1 ml of enzyme extract solution and 1 ml of H_2O_2 free phosphate buffer (0.2 M) was taken and set as blank. 3 ml of H_2O_2 -phosphate buffer was added to it and mixed gently. Peroxidase was extracted in prechilled distilled water. 1 ml of the extract was used which is centrifuged at 2000 rpm. 2 ml of phosphate buffer, 0.5 ml of 1% pyrogallol and 0.05 N H_2O_2 was used for the determination of peroxidase. The change in optical density was recorded at 425 nm.

Statistical analysis

The data obtained were analyzed statistically using SPSS software and test for significance (p = 0.05%).

RESULTS AND DISCUSSION

Grain yield

The different ESP levels significantly affected the grain yield of rice crop. The mean grain yield of different rice varieties

Table 1: Initial soil properties of experimental field.

ESP levels	pH	EC dS m^{-1}	N kg ha^{-1}	P kg ha^{-1}	K kg ha^{-1}	ESP (%)
8	8.25	0.73	194	19.87	179	8.94
16	8.70	1.03	192	18.35	176	15.7
24	8.74	1.10	191	17.92	174	23.6
32	9.66	1.16	189	17.41	173	31.8
40	10.1	1.25	183	16.73	170	39.6
48	10.3	1.47	181	16.26	165	48.3

at different sodicity levels ranged from 5434 to 947 kg ha^{-1} (Table 2). The highest grain yield was found at ESP 8 (5434 kg ha^{-1}) which is on par with ESP 16 (5204 kg ha^{-1}) followed by ESP 24, 32, 40 *ie.,* 4477, 2343, 1659 kg ha^{-1}, respectively. The lowest grain yield found at the ESP level of 48 (947 kg ha^{-1}). The grain yield was found to be significantly differ among the rice varieties. The highest grain yield was recorded in TRY 3 (4693 kg ha^{-1}) followed by CO 43 (3393 kg ha^{-1}), TRY 1 (3437 kg ha^{-1}), TRY 2 (3134 kg ha^{-1}), CSR 27 (3161 kg ha^{-1}) and the lowest yield was recorded in WP (2246 kg ha^{-1}). Different rice varieties exhibit the sodicity tolerance differently in terms of grain yield at different ESP levels. The TRY 3 variety gave atleast 50% (as compared to ESP 8) grain yield upto 32 ESP. However, 50% yield was recorded upto 16 ESP only in case of WP. However, 50% yield was recorded upto 24 ESP in case of TRY 1, CO 43, TRY 2 and CSR 27. The grain yield decreases with increasing sodicity levels and the reason for yield reduction is the dominance of sodium ions causes depletion of enzymatic activity in soil. Due to its great sensitivity of WP variety, the yield has substantially reduced at higher sodicity levels. The results are in consistent with (Gao *et al.,* 2007 and Singh *et al.,* 2016). The rice is notably sensitive to sodicity at the early seedling stage and significant losses in yield have been recorded as a result of high mortality and poor crop establishment.

Soil enzymes

Urease

The different ESP levels has significant effect on urease content and the mean value ranged from 14.1 to 5.2 µg NH_{4-}^{+}-N g^{-1} soil h^{-1} (Table 3). The highest urease content was observed at ESP 8 (14.1 µg NH_{4-}^{+}-N g^{-1} soil h^{-1}) which is on par with ESP 16 (14.1 µg NH_{4-}^{+}-N g^{-1} soil h^{-1}) followed by ESP 24 (13.8 µg NH_{4-}^{+}-N g^{-1} soil h^{-1}), ESP 32 (10.7 µg NH_{4-}^{+}-N g^{-1} soil h^{-1}) and ESP 40 (8.57 µg NH_{4-}^{+}-N g^{-1} soil h^{-1}). The lowest urease content was found at ESP 48 (5.24 µg NH_{4-}^{+}-N g^{-1} soil h^{-1}). At ESP 8, 16, 24 and 32 the urease content slowly decreases with increasing sodicity. At highest ESP, the urease activity was drastically reduced. At ESP 40 and 48 only 60.69% and 37.11% urease activity was observed when compared to ESP 8. Liang *et al.* (2007), reported that urease encourages the hydrolysis of nitrogen-containing organic carbon into ammonium. At increasing sodicity levels low hydrolysis process may be a reason for decreased urease activity in the soil. There is no significant difference found between the plots of different rice varieties which indicate that the varietal character doesn't affect the urease activity in soil. The interaction between the different ESP levels and different rice cultivated plots was also found to be non-significant.

Alkaline phosphatase

The data pertaining alkaline phosphatase was presented in Table 3. The alkaline phosphatase activity in soil decreases with increasing sodicity levels. The mean of APH activity at ESP 8, 16, 24, 32, 40 and 48 was found to be 156, 143, 94.2, 73.9, 52.7 and 25.9 µg PNP g^{-1} soil h^{-1}, respectively. The results were comparable with the works of Batra *et al.* (2010) which confirmed that and the alkaline phosphatase decreased with increase in salt concentration. At ESP 40 and 48 only 33.82% and 16.61% of APH activity found while compared to the activity at ESP 8. The reason for low phosphatase activity may be that increasing salt concentration which affects enzyme activity by influencing the concentration of inhibitors or activators in the soil solution and the effective concentration of the substrate (Dick *et al*., 2000). There is no significant differences observed between the plots of various rice varieties. Additionally, no significance difference was also found in the interaction between the various ESP levels and various rice varieties cultivated plots.

Dehydrogenase

The increasing sodicity levels had a significant effect on DHG content of soil. The highest dehydrogenase activity was found at ESP 8 (27.4 µg TPF g^{-1} soil 24 h^{-1}) followed by ESP 16 (25.5 µg TPF g^{-1} soil 24 h^{-1}). From ESP 24, 32 and 40 a low DHG activity was recorded *viz.,* 16.4, 10.2 and 2.60 µg TPF g^{-1} soil 24 h^{-1}, respectively (Table 4). The lowest activity was observed in ESP 48 (1.12 µg TPF g^{-1} soil 24 h^{-1}). Srivastava *et al.* (2014) reported that salt stress-induced soil physical qualities affect plants because DHG activity exhibited lower values in the case of sodic soil compared to

Table 2: Effect of different ESP levels on Grain yield (kg ha^{-1}) in different rice varieties.

ESP levels	TRY-1	CO-43	TRY-2	CSR-27	TRY-3	WP	Mean
8	5135	5725	4970	5255	6900	4620	5434
16	5055	5275	4900	5100	6885	4010	5204
24	4815	4480	4355	4705	6220	2285	4477
32	2465	2325	2157	2020	4035	1055	2343
40	1925	1860	1522	1043	2745	856.0	1659
48	961.0	955.0	901.0	841.0	1370	652.0	947.0
Mean	3393	3437	3134	3161	4693	2246	
	ESP levels		Variety	ESP×Variety			
SEm±	83.4		146	134			
CD	214*		375*	277*			

***Significant at (P≤0.05%).

Table 3: Effect of different sodicity levels on Urease content (µg NH_4^+-N released g^{-1} soil h^{-1}) and Alkaline phosphatase content (µg PNP g^{-1} soil h^{-1}) in soil at flowering stage of different rice varieties.

ESP	Urease							Alkaline phosphatase						
levels	TRY 1	C O 43	TRY 2	CSR 27	TRY 3	WP	Mean	TRY 1	C O 43	TRY 2	CSR 27	TRY 3	WP	Mean
8	14.1	14.1	14.1	14.1	14.2	14.1	14.1	157	156	155	158	155	154	156
16	14.1	14.0	14.1	14.0	14.1	14.1	14.1	141	145	147	141	142	144	143
24	13.8	13.9	13.8	13.8	13.8	13.7	13.8	94.9	96.3	92.7	95.4	93.8	92.1	94.2
32	10.6	10.7	10.6	10.8	10.7	10.8	10.7	73.9	74.2	75.2	73.6	74.3	72.5	73.9
40	8.39	8.62	8.53	8.79	8.53	8.53	8.57	52.7	51.9	53.7	52.9	51.2	53.6	52.7
48	5.23	5.16	5.25	5.32	5.29	5.20	5.24	26.7	26.8	25.7	24.8	25.9	25.3	25.9
Mean	11.0	11.1	11.1	11.1	11.1	11.1		91.0	91.6	91.5	90.8	90.4	90.2	
	ESP levels		Variety	ESP×Variety				ESP levels		Variety	ESP×Variety			
SEm±	0.522		0.163	0.148			SEm±	2.504		1.075	1.329			
CD	1.034*		NS	NS			CD	6.438*		NS	NS			

*Significant at (P≤0.05%); NS- Non Significant at P>0.05.

Table 4: Effect of different sodicity levels on dehydrogenase content (µg TPF g^{-1} soil 24 h^{-1}) and microbial biomass carbon (µg g^{-1}) in soil at flowering stage of different rice varieties.

ESP	Dehydrogenase							Microbial biomass carbon						
levels	TRY 1	C O 43	TRY 2	CSR 27	TRY 3	WP	Mean	TRY 1	C O 43	TRY 2	CSR 27	TRY 3	WP	Mean
8	27.6	27.3	27.2	27.2	27.9	27.4	27.4	236	235	235	233	236	234	235
16	25.6	25.6	25.4	25.2	25.8	25.5	25.5	232	234	234	232	233	231	233
24	16.6	16.3	16.3	16.2	16.7	16.1	16.4	193	193	194	195	192	194	193
32	10.2	10.1	10.2	10.1	10.2	10.1	10.2	175	174	173	176	174	173	174
40	2.60	2.58	2.64	2.57	2.63	2.59	2.60	166	168	165	167	167	166	167
48	1.12	1.14	1.12	1.11	1.13	1.10	1.12	159	158	158	159	157	157	158
Mean	13.9	13.8	13.8	13.7	14.1	13.8		193	193	193	194	193	193	
	ESP levels		Variety	ESP×Variety				ESP levels		Variety	ESP×Variety			
SEm±	0.41		0.58	0.58				3.58		2.19	1.45			
CD	0.82*		NS	NS				7.12*		NS	NS			

*Significant at (P≤0.05%); NS- Non Significant at P>0.05.

normal soil. The reason behind the decreased dehydrogenase activity under increasing sodicity levels may be alterations in the catalytic site of enzymes, ionization-induced conformational changes and salting out effect (Tejada *et al.,* 2006).

Microbial biomass carbon (MBC)

The results showed that the microbial biomass carbon decreases with increasing ESP levels (Table 4). The different ESP levels have significant effect on soil MBC and the mean value ranged from 235 to 158 µg g^{-1}. The highest soil MBC was observed at ESP 8 (235 µg g^{-1}) which is on par with ESP 16 (233 µg g^{-1}) followed by ESP 24 (193 µg g^{-1}), ESP 32 (174 µg g^{-1}) and ESP 40 (167 µg g^{-1}). The lowest soil MBC was found at ESP 48 (158 µg g^{-1}). Tripathi *et al.* (2006), observed that one of the causes of poor crop development in salt affected soils is likely to be a decline in MBC with increase in salt stress. However, the microbial activity in different rice varieties plots cultivated, interaction between ESP and different plots of rice varieties were found to be non-significant.

Activity of antioxidant enzymes in plants

Catalase activity

The different ESP levels have significant effect on catalase activity. The mean of Catalase (CAT) activity at different sodicity levels *viz.,* ESP 8, 16, 24, 32, 40 and 48 was found to be 54.0, 55.3, 56.7, 58.9, 60.6 and 59.6 µg H_2O_2 $g^{-1}min^{-1}$, respectively (Table 5). The CAT activity increases with increasing sodicity levels. However, at higher ESP levels the activity was slightly reduced. It is in agreement with the studies of Tripathi *et al.* (2018). In case of different rice variety, the highest CAT activity was recorded in CO 43 followed by TRY 3, TRY 1, CSR 27, TRY 2 and the lowest activity was found in WP. The results are in accordance with Geetha *et al.* (2022), where the tolerant rice varieties recorded higher CAT activity than susceptible varieties. In interaction between the ESP levels and variety, the tolerant varieties CAT increases only upto ESP 40 and slightly decreased at ESP 48. However, in WP, the CAT activity increased upto 32 ESP only and then after it drastically reduced. Tolerable rice varieties at flowering phase of observation produced considerably more CAT activity upto ESP 40 than susceptible varieties which is also reflected in yield. Increase in CAT activity helps to achieve atleast 50% of yield upto ESP 32.

Peroxidase activity

Peroxidase **(**POX) activity increases with increasing sodicity levels in tolerant varieties. Different ESP level has a significant effect on POX activity. The different ESP levels *viz.,* ESP 8, 16, 24, 32, 40 and 48 recorded the peroxidase activity of 10.6, 11.5, 11.9, 11.7, 11.5 and 11.1 $min^{-1}g^{-1}$, respectively (Table 5). Among the rice varieties, TRY 3 recorded the highest activity followed by TRY 1, CO 43, TRY 2, CSR 27 and the lowest in WP. Interaction between ESP levels and different rice varieties indicates that in rice variety

Table 5: Effect of different ESP levels on catalase (µg H_2O_2 g^{-1} min^{-1}) and Peroxidase (430 nm min^{-1} g^{-1}) of different rice varieties.

ESP	Catalase							Peroxidase						
level	TRY 1	C O 43	TRY 2	CSR 27	TRY 3	WP	Mean	TRY 1	C O 43	TRY 2	CSR 27	TRY 3	WP	Mean
8	51.0	64.6	51.0	51.0	55.3	51.0	54.0	12.2	12.0	8.80	7.40	14.4	8.60	10.6
16	52.7	66.3	51.0	53.6	56.1	51.9	55.3	12.8	12.4	9.40	8.0	14.8	11.6	11.5
24	53.6	67.2	52.7	55.3	57.8	53.6	56.7	13.0	12.8	10.6	9.8	15.2	10.0	11.9
32	56.1	68.9	55.3	57.0	62.1	54.4	58.9	13.8	13.0	11.0	10.4	15.4	6.80	11.7
40	60.4	72.3	57.8	57.0	64.6	51.4	60.6	13.7	12.8	10.4	10.2	16.0	5.80	11.5
48	59.1	71.1	56.7	55.5	67.2	48.1	59.6	13.2	12.4	10.2	10.1	15.8	4.60	11.1
Mean	55.5	68.4	54.1	54.9	60.5	51.7		13.1	12.6	10.1	9.32	15.3	7.90	
	ESP levels		Variety	ESP×Variety				ESP levels		Variety	ESP×Variety			
SEm±	0.75		2.34	0.80				0.24		0.16	0.13			
CD	1.93*		6.02*	1.66*				0.61*		0.41*	0.28*			

*Significant at (P≤0.05).

TRY 3, the POX activity increases upto ESP 40 and decreases at ESP 48. In case of TRY 1, CO 43, TRY 2 and CSR 27 the POX activity increased upto ESP 32 and slowly declines at ESP 40 and 48 which are also reflected in grain yield. At ESP 32, increased POX activity enables us to reach up to 50% of yield. In WP initially at ESP 8 and 16 the POX activity increases and from ESP 24 to 48 the activity was decreasing at increasing sodicity levels. The percentage rise was higher in tolerant cultivars, demonstrating that they are naturally able to survive the stress situation. Comparable results were in accordance with Upadhay and Kumar (2022) stating that peroxidase activity was one among useful parameter for the identification of tolerant and susceptible genotypes.

CONCLUSION

The interaction between soil enzymatic activity and different rice cultivated plots was found to be non-significant. However, soil enzyme activity and soil MBC decreases with increasing ESP levels. A reverse trend was observed in antioxidant enzymes. The genotypes TRY 3 followed by TRY 1, CO 43, TRY 2 and CSR 27 significantly recorded higher catalase activity upto ESP 40 and peroxidase upto ESP 32 and decreases eventually after that. The increase in catalase and peroxidase increases the tolerance mechanism and produces a reasonable yield in tolerant varieties at higher sodicity levels. In view of antioxidant enzymes and grain yield, overall TRY 3 outperformed among other tolerant rice varieties studied and 50% of yield can be achieved upto ESP 32. At higher sodicity levels the antioxidant enzymes in tolerant varieties increased gradually and expose its tolerance capacity. So, improving the soil enzymatic activity, soil MBC and plant antioxidant enzymes will further increase the growth and yield of rice under higher sodicity levels.

ACKNOWLEDGEMENT

The authors are thankful to scientists who helped technically in analysis.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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