Int.J.Curr.Microbiol.App.Sci (2018) *7*(1): 2640-2647

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.701.315

Activity of Soil Urease, Phosphatase and Dehydrogenase as Influenced by Various Sources of Zinc in Rice (*Oryza sativa* L.)

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Keywords

Nano zinc, Bio zinc, Rice, Soil application, Enzymes

Article Info

Accepted:
20 December 2017
Available Online:
10 January 2018

A B S T R A C T

A field experiment was conducted during *kharif*, 2015 at college farm, college of agriculture, Rajendranagar, Hyderabad to study the influence of various sources of zinc on soil enzyme activity. The experiment was laid out in Randomized Block Design with 12 treatments and 3 replications. The results of the experiments revealed that the highest urease activity at harvest was recorded in the treatment RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (370.3µg NH_4^+ released g^{-1} soil 2 hr^{-1}), similarly in the acid and alkaline phosphatase activity highest value was seen in RDF+ Soil application of bio zinc@30 kg ha^{-1} (106.0, 183.7µg of *p*-nitrophenol g^{-1} soil hr^{-1}). The highest dehydrogenase activity was recorded in RDF+ Soil application of bio zinc@30 $kgha^{-1}$ (84.8 µg TPF produced g soil d^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (80.8 µg TPF produced g soil d^{-1}). All the three enzyme activities were increased from the vegetative to panicle emergence stage and later showed decrease at harvest stage.

Introduction

Rice (*Oryza sativa L.)* is an important staple food crop among all the cereals. About 90 % of rice grown and consumed in south and south East Asia. In some parts of the world consumption of rice is as high as 990 g per person per day (Sharma *et al.*, 2015). India ranks first in the world in terms of area of rice cultivation with 44.6 m ha and second in productivity of 2.96 t ha^{-1}.In Telangana state, rice is grown in an area of 17 lakh $ha^{-1}$with a production of 64 lakh metric tons with a productivity of 3.6 t ha^{-1} (India Stat., 2015-2016).

In India, zinc is considered as the fourth important yield limiting nutrient after nitrogen, phosphorus and potassium respectively. Zinc deficiency affects one third of the world's population.

In India, 47% of the soils are Zn deficient. Critical limit of a nutrient in soils refers to a level below which the crops will readily respond to its application. This critical limit varies with soil, crops and varieties. Critical limit of Zn for rice was 0.74±0.18 ppm across the soils and indifferent agro-ecological regions of India (Muthukumararaja and Sriramachandrasekharan 2012). Hence,

application of zinc fertilizers is essential in keeping sufficient amount of available zinc in soil solution, maintaining adequate zinc transport to seeds and for increases in the crop yield. Foliar or combined soil + foliar application of fertilizers under field conditions have proved to be highly effective and can be a practical way to maximize the zinc accumulation and uptake in grains.

Regarding the importance of Zn, it is an essential element for all organisms. In its oxidized Zn (II) form, it acts as a catalytic or structural cofactor in a large number of enzymes and regulatory proteins (Maret 2009). Many plant processes are regulated by Zn containing enzymes such as CO_2 fixation, maintenance of biological membranes, protein synthesis, auxin synthesis and formation of pollen grains. The efficiency of applied $ZnSO_4$ is only 1 to 4% and most of the applied zinc is rendered unavailable to plants due to many factors such as leaching, fixation (Nair *et al.,* 2010). Hence it is essential to minimize the nutrient losses in fertilizer application, increase the crop yield through the exploitation of new applications with the help of nano technology and nano meterials.

Nano fertilizers have unique physicochemical properties and the potential to boost the plant metabolism (Giraldo *et al.,* 2014). The nano fertilizers or nano encapsulated nutrients might have the properties that are effective to crops, release the nutrients on demand, controlled release of chemical fertilizers that regulate the plant growth and enhanced target activity (DeRosa *et al.,* 2010). Literally very little information exist on the application of nano zinc both in chemical and bioforms applied to soil and foliar application of these materials on rice crop under field conditions. Considering the deficiency status of Zn in soil and its importance, an attempt has been made to evaluate the effects of various methods of Zn application on key enzymes in soil.

Materials and Methods

A field experiment was conducted during *kharif,* 2015 at College Farm, College of Agriculture, PJTSAU. Experiment was laid out in Randomized Block Design with 12 treatments and 3 replications. The rice variety used was MTU-1010. The treatments were viz., T1-Control(no fertilizers were applied), T2- RDF@ N,P_2O_5,K_2O@120:60:40 kg ha^{-1}, T3-RDF+Soil application of $ZnSO_4$@25kgha^{-1} at transplanting, T4 and T5- RDF +Soil application of nano Zn @10 kg ha^{-1} and 15 kg ha^{-1}, T6 and T7- RDF +Soil application of bio Zn @15 kg $ha^{-1}$and 30 kg ha^{-1} at transplanting, T8-RDF +foliar application of 0.2 % as $ZnSO_4$ at tillering and panicle emergence stage, T9 and T10-RDF +foliar application of 1 ml l^{-1} and 2 ml l^{-1} as nano zinc at tillering and panicle emergence stage, T11 and T12 -RDF +foliar application of 1.5ml l^{-1} and 3ml l^{-1} as bio zinc at tillering and panicle emergence stage. The study was taken up on a Vertisol (pH 8.24, EC:0.74dSm^{-1}), low in organic carbon (0.42%), low in Nitrogen (242 kg ha^{-1}), high in available Phosphorus (92 kg P_2O_5 ha^{-1}) and high in available Potassium(376 kg K_2O ha^{-1}). The DTPA extractable zinc was 0.3 $mgkg^{-1}$.

Application of fertilizers

The products i.e., nano zinc and bio zinc formulations were obtained from M/S. Prathishta industries, Alwal, Secunderabad. These are being manufactured by the firm. The nano zinc soil and foliar formulation had Zn content of 40 mg kg^{-1} and bio zinc soil and foliar formulation contains 3% Zn. Along with 16% organic carbon.

Assay of enzyme activity in soil

Soil samples of each treatmental plot were collected at tillering stage, panicle initiation, seed filling and at harvest stage, and were immediately stored in polythene bags. The

soils were preserved and stored at 5°C in a refrigerator until analysis. These samples were utilized for the assay of soil enzyme activity.

Methods employed for determination enzyme activity in soil

Enzyme	Method employed
Urease	Tabatabai and Bremner (1972) µg of NH^{+4} released g^{-1} $2h^{-1}$
Acid & Alkaline Phosphatase	Tabatabai and Bremner (1969) p-nitrophenol released g^{-1} soil h^{-1}
Dehydrogenase	Cassida *et al.*, (1964). µg of TPF g^{-1} soil day^{-1}

The recorded data were subjected to statistical analysis using the analysis of variance technique for randomized block design as suggested by Panse and Sukhame (1978).

Results and Discussion

The results obtained from the present investigation on soil enzyme activity is presented in the following heads.

Urease activity

Soil urease plays a major role in catalysis of the hydrolysis of urea to ammonical form, which will be subsequently oxidized by nitrifiers to nitrate form, which increases the utilization rate of nitrogen fertilizer. Effect of different sources of zinc on the activity of soil urease (µg NH_4^+ released g^{-1} soil 2 hr^{-1}) at different growth stages are presented in Table 1. The urease activity showed an increasing trend with the age of the crop. It increased from tillering stage to panicle emergence stage, exhibited highest activity at panicle emergence stage and there after the activity decreased at maturity. Similar results were reported by Senthil Kumar *et al.*, (2000) at different growth stages of rice.

At 30DAT the highest urease activity was observed in the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} (395 µg NH_4^+ released g^{-1} soil 2 hr^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (389.6 µg NH_4^+ released g^{-1} soil 2 hr^{-1}). The lowest urease activity was observed in control (136.0 µg NH_4^+ released g^{-1} soil 2 hr^{-1}) which was followed by RDF+ foliar application of 0.2% zinc as $ZnSO_4$ (169.3 µg NH_4^+ released g^{-1} soil 2 hr^{-1}) which was on par with RDF+ foliar spray of 1ml l^{-1} as nano zinc (171.0 µg NH_4^+ released g^{-1} soil 2 hr^{-1}).

At 60DAT there was a maximum increase in urease activity and the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} recorded the highest activity (419.3µg NH_4^+ released g^{-1} soil 2 hr^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (406.0 µg NH_4^+ released g^{-1} soil 2 hr^{-1}), RDF+ Soil application of bio zinc@ 15 kg ha^{-1} (389.6 µg NH_4^+ released g^{-1} soil 2 hr^{-1}). At harvest there was a decrease in urease activity among all the treatments and the highest value was seen in RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (370.3µg NH_4^+ released g^{-1} soil 2 hr^{-1}). The lowest was recorded in control (93.4 8 µg NH_4^+ released g^{-1} soil 2 hr^{-1}). Similar results were also reported by Ramlakshmi (2011) at different growth stages of rice.

Acid and alkaline phosphatase activity

Phosphatases are broad groups of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid. In soil ecosystems, these enzymes are believed to play critical roles in P cycles (Speir and Ross,

1978) as evidence shows that they are correlated to P stress and plant growth. The effect of different sources of zinc on the activity of acid and alkaline phosphatase (µg of *p*-nitrophenol g^{-1} soil hr^{-1}) is presented in Table 2.

Acid phosphatase activity

At 30DAT the highest acid phosphatase activity was observed in the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} (135.2 µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (129.8 µg of *p*-nitrophenol g^{-1} soil hr^{-1}).

The lowest acid phosphatase activity was observed in control (72.3 µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which was on par with RDF+ foliar application of 0.2% $ZnSO_4$ spray (72.3 µg of *p*-nitrophenol g^{-1} soil hr^{-1}).

Table.1 Effect of various sources of zinc on soil urease activity (µg of NH^{+4} g^{-1} soil $2h^{-1}$)

S. No	Treatment	30DAT Tillering stage	60DAT Panicle emergence stage	90DAT Grain filling stage	Harvest
T1	**Control (no fertilizers were applied)**	136.0	187.3	145.3	93.4
T2	**Recommended dose of N:P_2O_5:K_2O @120:60:40 Kg ha^{-1}**	286.0	309.2	282.8	258.0
T3	**RDF +Soil application of ZnSO4 @25Kg ha^{-1} at transplanting**	389.6	406.0	393.9	370.3
T4	**RDF +Soil application of nano zinc as impregnated granules @10kg ha^{-1} at transplanting**	296.0	326.3	294.0	239.0
T5	**RDF +Soil application of nano zinc as impregnated granules @15kg ha^{-1} at transplanting**	324.3	365.5	265.8	209.1
T6	**RDF +Soil application of bio zinc @15 kg ha^{-1} at transplanting**	338.9	389.6	333.9	300.8
T7	**RDF +Soil application of bio zinc @30 kg ha^{-1} at transplanting.**	395.0	419.3	369.7	321.0
T8	**RDF +Foliar spray of 0.2% as $ZnSO_4$**	169.3	216.0	177.6	121.9
T9	**RDF +Foliar spray of 1ml l^{-1} as nano zinc**	171.0	252.1	213.0	162.5
T10	**RDF +Foliar spray of 2ml l^{-1} as nano zinc**	211.0	272.0	215.7	197.8
T11	**RDF +Foliar spray of 1.5ml l^{-1} as bio zinc**	227.8	282.6	256.8	198.9
T12	**RDF +Foliar spray of 3ml l^{-1} as bio zinc**	229.6	300.6	276.6	204.7
	SE(m) ±	16.9	17.2	16.4	12.6
	CD (P=0.05)	50.1	51.0	48.4	37.3

Table.2 Effect of various sources of zinc on Acid and Alkaline phosphatase activity in soil (µg of *P- Nitrophenol* g soil^{-1} h^{-1})

S. No	Treatment	Acid phosphatase activity (µg of *P- Nitrophenol* g Soil^{-1} h^{-1})				Alkaline phosphatase activity (µg of *P- Nitrophenol* g Soil^{-1} h^{-1})			
		30 DAT	60 DAT	90 DAT	Harvest	30 DAT	60 DAT	90 DAT	Harvest
T1	Control (no fertilizers were applied)	72.3	97.6	70.0	65.9	87.0	151.0	102.9	68.7
T2	Recommended dose of N:P_2O_5:K_2O @120:60:40 Kg ha^{-1}	90.4	122.8	100.8	83.9	140.4	190.6	160.9	101.8
T3	RDF +Soil application of ZnSO4 @25Kg ha^{-1} at transplanting	129.8	141.3	130.9	98.8	190.0	221.1	200.0	178.0
T4	RDF +Soil application of nano zinc as impregnated granules @10kg ha^{-1} at transplanting	96.7	128.0	109.0	90.9	148.7	198.0	167.0	105.1
T5	RDF +Soil application of nano zinc as impregnated granules @15kg ha^{-1} at transplanting	107.9	130.7	114.0	90.8	176.0	197.0	180.6	150.0
T6	RDF +Soil application of bio zinc @15 kg ha^{-1} at transplanting	109.0	139.0	125.1	96.8	187.4	201.6	190.5	164.6
T7	RDF +Soil application of bio zinc @30 kg ha^{-1} at transplanting.	135.2	167.0	149.0	106.0	206.1	245.0	219.0	183.7
T8	RDF +Foliar spray of 0.2% as $ZnSO_4$	72.3	97.9	70.0	65.9	90.0	156.9	112.1	81.4
T9	RDF +Foliar spray of 1ml l^{-1} as nano zinc	106.0	130.9	114.0	90.8	94.9	165.0	123.0	83.8
T10	RDF +Foliar spray of 2ml l^{-1} as nano zinc	84.0	114.9	85.9	74.8	109.0	170.9	139.2	94.1
T11	RDF +Foliar spray of 1.5ml l^{-1} as bio zinc	88.6	118.0	93.3	76.9	120.0	180.9	141.0	97.0
T12	RDF +Foliar spray of 3ml l^{-1} as bio zinc	90.0	121.0	98.8	81.0	123.0	185.3	152.1	100.0
	SE(m) ±	5.6	4.0	6.9	5.1	11.4	6.5	13.3	8.0
	CD (P=0.05)	16.5	11.8	20.4	15.2	33.8	19.3	39.9	23.3

Table.3 Effect of various sources of zinc on dehydrogenase activity in soil (µg of TPF g soil^{-1} Day^{-1})

S. No	Treatment	30DAT Tillering stage	60DAT Panicle emergence stage	90DAT Grain filling stage	Harvest
T1	Control (no fertilizers were applied)	30.2	50.0	36.0	30.2
T2	Recommended dose of N:P_2O_5:K_2O @120:60:40 Kg ha^{-1}	56.9	80.0	67.7	50.7
T3	RDF +Soil application of ZnSO4 @25Kg ha^{-1} at transplanting	81.9	134.3	104.1	80.8
T4	RDF +Soil application of nano zinc as impregnated granules @10kg ha^{-1} at transplanting	60.2	88.6	72.2	57.2
T5	RDF +Soil application of nano zinc as impregnated granules @15kg ha^{-1} at transplanting	65.1	91.2	83.6	60.1
T6	RDF +Soil application of bio zinc @15 kg ha^{-1} at transplanting	76.8	122.0	99.2	70.2
T7	RDF +Soil application of bio zinc @30 kg ha^{-1} at transplanting.	86.5	145.8	114.9	84.8
T8	RDF +Foliar spray of 0.2% as $ZnSO_4$	39.2	71.0	55.0	33.9
T9	RDF +Foliar spray of 1ml l^{-1} as nano zinc	41.2	72.9	46.1	40.0
T10	RDF +Foliar spray of 2ml l^{-1} as nano zinc	40.9	79.7	57.7	40.8
T11	RDF +Foliar spray of 1.5ml l^{-1} as bio zinc	46.0	70.9	59.8	43.0
T12	RDF +Foliar spray of 3ml l^{-1} as bio zinc	50.6	82.0	64.9	45.1
	SE(m) ±	2.5	3.3	4.3	5.8
	CD (P=0.05)	7.6	10.0	12.8	17.3

At 60 DAT stage there was a maximum increase in acid phosphatase activity and the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} recorded the highest activity (167.0 µg of *p*-nitrophenol g^{-1} soil hr^{-1}). There was a gradual decrease seen in acid phosphatase activity at 90DAT and the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} (149.0 µg of *p*-nitrophenol g^{-1} soil hr^{-1}) recorded the highest acitivity and was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (130.9 µg of *p*-nitrophenol g^{-1} soil hr^{-1}).

At harvest there was a much decrease in acid phosphatase activity among all the treatments and the highest value was seen in RDF+ Soil application of bio zinc@30 kgha^{-1} (106.0 µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (98.8 µg of *p*-nitrophenol g^{-1} soil hr^{-1}), RDF+ Soil application of bio zinc@ 15 kg ha^{-1} (96.8 µg of *p*-nitrophenol g^{-1} soil hr^{-1}). The lowest was recorded in control (65.9 µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which was on par with RDF+ foliar spray of 0.2% $ZnSO_4$ (65.9 µg of *p*-nitrophenol g^{-1} soil).

Alkaline phosphatase activity

At maximum tillering the highest alkaline phosphatase activity was observed in the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} (206.1 µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (190.0µg of *p*-

nitrophenol g^{-1} soil hr^{-1}), RDF+ Soil application of bio zinc@15 kg ha^{-1} (187.4µg of *p*-nitrophenol g^{-1} soil hr^{-1}), RDF+ Soil application of nano zinc@15 kg ha^{-1} (176.0µg of *p*-nitrophenol g^{-1} soil hr^{-1}).

At panicle emergence stage there was a maximum increase in alkaline phosphatase activity and the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} recorded the highest activity (245.0µg of *p*-nitrophenol g^{-1} soil hr^{-1}). This treatment was followed by RDF+ Soil application of $ZnSO_4$@25kg ha^{-1} (221.1µg of *p*-nitrophenol g^{-1} soil hr^{-1}), RDF+ Soil application of bio zinc@ 15 kg ha^{-1}(201.6µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which were on par with each other. The lowest activity was recorded in control (151.0 µg of *p*-nitrophenol g^{-1} soil hr^{-1}). There was a gradual decrease seen in acid phosphatase activity at 90DAT.

At harvest there was a much decrease in alkaline phosphatase activity among all the treatments and the highest value was seen in RDF+ Soil application of bio zinc@30 kg ha^{-1} (183.7µg of *p*-nitrophenol g^{-1} soil hr^{-1}) which was on par with RDF+ Soil application of

$ZnSO_4$@25 kg ha^{-1} (178.0 µg of *p*-nitrophenol g^{-1} soil hr^{-1}), RDF+ Soil application of bio zinc@ 15 kg ha^{-1} (164.6 µg of *p*-nitrophenol g^{-1} soil hr^{-1}). The lowest was recorded in control (68.7µg of *p*-nitrophenol g^{-1}soil hr^{-1}).

Dehydrogenase activity

Dehydrogenase is considered as an indicator of overall microbial activity because it has intracellular activity in all living microbial cells and it is linked with microbial respiratory process. The dehydrogenase activity is commonly used as an indicator of biological activity in soils (Burns, 1978). Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil microorganisms and closely related to the type of soil. The data on effect of sources of zinc on activity of dehydrogenase (µg TPF produced g soil d^{-1}) is presented in Table 3.

At 30DAT the highest dehydrogenase activity was observed in the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} (86.5µg TPF produced g soil d^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (81.9 µg TPF produced g soil d^{-1}). These treatments were followed by RDF+ Soil application of bio zinc@15 kg ha^{-1} (76.8 µg TPF produced g soil d^{-1}). The lowest dehydrogenase activity was observed in control (30.2 µg TPF produced g soil d^{-1}) which was on par with RDF+ foliar application of 0.2% $ZnSO_4$ spray (39.2 µg TPF produced g soil d^{-1}).

At 60DAT there was a maximum increase in dehydrogenase activity and the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} recorded the highest activity (145.8 µg TPF produced g soil d^{-1}).

There was a gradual decrease in dehydrogense activity at 90DAT and the treatment receiving RDF+ Soil application of biozinc@30 kg ha^{-1} (114.9 µg TPF produced g soil d^{-1}) recorded the highest activity and was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1} (104.1µg TPF produced g soil d^{-1}).

At harvest there was a much decrease in dehydrogenase activity among all the treatments and the highest value was seen in RDF+ Soil application of bio zinc@30 kgha^{-1}(84.8 µg TPF produced g soil d^{-1}) which was on par with RDF+ Soil application of $ZnSO_4$@25 kg ha^{-1}(80.8 µg TPF produced g soil d^{-1}), RDF+ Soil application of bio zinc@ 15 kg ha^{-1}(70.2 µg TPF produced g soil d^{-1}). The lowest was recorded in control (30.2 µg TPF produced g soil d^{-1}). Similar to urease and phosphatase the dehydrogenase activity increased with the age of the crop and attained maximum at 60 DAT and decreased at harvest. Similar results were also reported by Rai and Yadav (2011). The increase in dehydrogense activity was attributed due to increase in population of anaerobic microorganism in submerged soils. There was a

shift in soil micro flora from aerobic of facultative and obligatory anaerobic ones after the soil is flooded. The shift from aerobic to anaerobic microorganism was found to increase the dehydrogenase activity.

It has been observed that the treatment receiving the soil application of biozinc@30 kg ha^{-1}have lead to an increased activity of enzymes. Addition of trace metals like zinc to soil may influence microbial proliferation and enzyme activity possibly leading to an increase in rates of biochemical process in the soil environment. However when application of abnormally higher concentration rate they could cause an increase in inhibition of enzyme activity. It has been observed that trace elements as activation of enzyme in soil varies with the soil, the concentration and the form of added trace element on the enzyme assay.

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How to cite this article:

Apoorva, M.R., P. Chandrasekhar Rao, G. Padmaja and Subhash Reddy, R. 2018. Activity of Soil Urease, Phosphatase and Dehydrogenase as Influenced by Various Sources of Zinc in Rice (*Oryza sativa* L.). *Int.J.Curr.Microbiol.App.Sci*. 7(01): 2640-2647.
doi: https://doi.org/10.20546/ijcmas.2018.701.315