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ORIGINAL ARTICLE

Zinc Fertilization Effect on Soil Microbial Activities

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ABSTRACT
Fertilization of crop with zinc (Zn) has received considerable attention in recent year due to world-wide spread of its deficiency in soil and also due to malnutrition, especially in developing countries. Soil samples were collected from rice field at three different stages of rice growth i.e. maximum tillering stage, panicle initiation stage and harvesting stage. Soil enzymes regulate ecosystem functioning and in particular play a key role in nutrient cycling. The Zn content in root was highest in root of rice plant followed by grain. The largest effect on grain Zn was observed by combination of soil and foliar applications. The Zn content was highest in T7 followed by T6 over untreated control plot. Allocation of Zn is necessary for enriching grain with Zn content. The data revealed that application of zinc fertilizers has resulted in lowering alkaline phosphatase enzyme activity in soil. Alkaline phosphatase enzyme activity was increases first then started gradually deceasing in all 3 stages of plant growth of rice. it has been found that proliferation of bacteria was significantly increasing in all the treatments as compared to untreated control. Plant was able to produce higher root exudates when zinc was applied as both foliar and in soil which had stimulatory effect on bacterial population. that plant was able to produce higher root exudates when Zinc was applied as both foliar and in soil which turn had stimulatory effect on actinomycetes population. plant was able to produce higher root exudates when zinc was applied as both foliar and in soil which turn had stimulatory effect on fungi population. As dehydrogenase is an enzyme that occurs in all viable microbial cells, these enzymes function as a measurement of the metabolic state of soil microorganisms. Soil microorganisms such as bacteria, actinomycetes, fungi, are involved in several processes that influence soil quality and assessing the effect of management on soil microorganisms. The increase in microbial biomass carbon can be attributed to the fact that incorporation of zinc resulted in increase in the proliferation of microbial population in soil system which ultimately resulted in increase in microbial biomass carbon content in soil.
Keywords: microorganisms, actinomycetes, dehydrogenase, Fertilization

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INTRODUCTION

Zinc is one of the important micronutrients essential for plants, animals and human health. It is needed in very small amount but from the nutritional point of view, it is indispensable like any other essential nutrients. Among the micronutrients, Zn is the most researched and talked about nutrients not only in India, but in the world. Fertilization of crop with zinc (Zn) has received considerable attention in recent year due to world-wide spread of its deficiency in soil and also due to malnutrition, especially in developing countries. Almost half of the soils in the world are deficient in available zinc (25). Indian soils are generally low in zinc. The field scale Zn deficiency in rice in *tarai* soils was first reported by Nene in 1966 in India. Zn plays an important role in maintaining the structure and function of large number of macromolecules and is also found responsible for controlling over 300 enzymatic reactions (28). The use of chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the complex biogeochemical cycles (27).

MATERIAL AND METHODS

The experiment was carried out at Central Research Farm, Gayeshpur, Nadia under A.I.C.R.P. on Micronutrients. The latitude and longitude of the experiment location are 22.96^{0}N and 88.49^{0}E, respectively. Swarna mashuri variety of rice is used in this experiment. Fields were divided into 24 plots and water channel were made after every 8 plot. Area of individual plot is 3m x 26 m. The experiment consisted of the following eight treatments: **T1**= No Zn, **T2**= Soil application of Zn @ 2.5 Kg/ha,**T3**= Soil application of Zn @ 5 Kg/ha,**T4**= Soil application of Zn @ 10 Kg/ha,**T5**= Soil application of Zn @ 2.5 Kg/ha + one foliar spray of $ZnSO_4.7H_2O$ @ 0.5% at booting stage, **T6**= Soil application of Zn @ 5 Kg/ha + one foliar spray of $ZnSO_4.7H_2O$ @ 0.5% at booting stage, **T7**= Soil application of Zn @ 10 Kg/ha + one foliar spray of $ZnSO_4.7H_2O$ @ 0.5% at booting stage, **T8**= Two foliar application of $ZnSO_4.7H_2O$ @ 0.5% at maximum tillering stage and at booting stage. To assessing the biochemical and microbial properties of soil standard methods were used (Table 1) and available Zn content in the soil was determined with the help of atomic absorption spectrophotometer (16). Soil samples were collected from rice field at three different stages of rice growth i.e. maximum tillering stage, panicle initiation stage and harvesting stage.

Table 1: Soil Parameters

Soil Parameters	**Methodologies**
Microbial Biomass Carbon	Chloroform fumigation extraction
Enumerate the colony forming units (CFU) of total bacteria, actinomycetes, fungi, nitrifying bacteria, ammonifying bacteria, aerobic non-symbiotic N_2-fixing bacteria	Serial dilution and pour plate technique
Phosphatase enzyme	
Dehydrogenase enzyme	

RESULTS AND DISCUSSION

The dissertation work deals with zinc fertilization and its effects microbial activities in soil. Soil enzymes regulate ecosystem functioning and in particular play a key role in nutrient cycling. In this review, summarized the data of selected enzymes such as dehydrogenase, phosphatase and microbial biomass carbon in the ecosystem. We also highlight the effect of zinc fertilizer on total population of Bacteria, Actinomycetes and Fungi present in soil.

Available Zinc status of soil and plant

The data on available Zn has been summarised in the table 2. A perusal of data revealed that application of zinc fertilizers had resulted in increasing the available zinc content of soil. The incorporation of $ZnSO_4.7H_2O$ as soil or as foliar or both has significant effect on available zinc content of soil. The initial available Zn content in soil ranges to 1.97 to 2.28 mg kg^{-1}. The increase in available Zn was highest when Soil application of Zn @ 10 kg ha^{-1} + one foliar spray of $ZnSO_4.7H_2O$ @ 0.5% at booting stage. The increase was 39.31 % than the untreated control plot. Available Zn content was increasing when Zn application @ 2.5, 5.0, 10.0 kg ha^{-1} respectively at all stages of plant growth. At PI stage, the increase in available Zn was highest in T7 (54.57 % over control plot) followed by T6 (48.17 % over control plot). At harvesting stage, the increase in available Zn was highest in T7 (48.69 % over control plot) followed by T6 (32.53 % over control plot). The increase in zinc by both soil+foliar application than only soil and only foliar spray may be due to the application of zinc fertilizer viz. $ZnSO_4.7H_2O$ which contain about 40 percent zinc by mass. The mean value of zinc content of plant parts has been given in table 3. The Zn content in root was highest in root of rice plant followed by grain. The largest effect on grain Zn was observed by combination of soil and foliar applications. The Zn content was highest in T7 followed by T6 over untreated control plot. Allocation of Zn is necessary for enriching grain with Zn content.

Table 2: available zinc content in soil(mg kg^{-1})

Treatment	Initial	Maximum tillering stage	Panicle initiation stage	Harvesting stage
T1	1.97	2.90	3.28	3.75
T2	2.26	3.28	3.46	3.69
T3	2.28	3.66	3.99	3.95
T4	2.32	3.82	4.35	5.17
T5	2.08	3.35	4.45	4.79
T6	2.22	3.81	4.86	4.86
T7	2.14	4.04	5.08	5.43
T8	2.18	3.57	3.68	4.97
S.E m	0.03	0.14	0.20	0.13
LSD (P=0.05)	0.09	0.40	0.57	0.38
C.V (%)	2.62	6.71	8.35	5.02

Table 3: zinc content in rice plant in different stage (mg kg^{-1})

	Maximum tillering stage		Panicle initiation stage		Harvesting stage		
Treatment	Root	Shoot	Root	Shoot	Root	Shoot	Grain
T1	47.55	32.33	50.88	34.90	48.65	35.52	40.52
T2	51.60	34.30	52.33	35.52	52.73	36.17	41.17
T3	53.28	33.93	56.06	35.12	55.33	35.82	40.82
T4	53.93	38.13	57.61	39.93	56.96	36.87	41.46
T5	51.98	40.03	59.10	42.60	53.94	41.60	45.76
T6	54.17	38.97	64.29	41.95	56.27	42.85	46.75
T7	55.15	39.40	64.51	42.15	57.21	43.25	47.25
T8	52.52	37.33	61.14	38.38	53.82	39.54	44.44
S.E m	1.22	0.93	1.19	0.97	0.83	0.66	0.68
C.D	3.49	2.66	3.41	2.77	2.39	1.89	1.96
C.V (%)	**4.01**	**4.36**	**3.53**	**4.31**	**2.66**	**2.93**	**2.72**

Phosphatase enzyme activity:

The data on phosphatase enzyme has been summarised in the table 4. A perusal of data revealed that application of zinc fertilizers has resulted in lowering acid phosphatase enzyme activity in soil. At maximum tillering stage,phosphatase enzyme activity was lowest in T7 (6.33% decrease over control) followed by T4 (4.82decrease over control) as compare to untreated control plot. The decrease in phosphatase activity was highest when Zn was applied in soil along with foliar at higher doses in panicle initiation stage. Statistically T7, T6, T4 has similar value which is lower than control plot. At harvesting stage, lowest phosphatase value found in T7(9.67% decrease over control) followed by T8 (5.49 % decrease over control).At harvesting stage, lowest value of phosphatase was found in T7 which is 8.69 % lower than control.Enzymes containing sulfo-hydryl groups such as phosphatase are usually deactivated by metals ions through the reactions between the functional group and metal, leading to the formation of a metal sulfide (14). [12] described that that acid phosphatase was deactivated by Cu and Zn, with a greater inhibition of the enzyme activity by Cu than by Zn.The data revealed that application of zinc fertilizers has resulted in lowering alkaline phosphatase enzyme activity in soil. Alkaline phosphatase enzyme activity was increases first then started gradually deceasing in all 3 stages of plant growth of rice. The similar result was found in [10].

Table 4: Phosphatase enzyme activity in soil

Treatment	Acid phosphatase			Alkaline phosphatase		
	Maximum tillering stage	Panicle initiation stage	Harvesting stage	Maximum tillering stage	Panicle initiation stage	Harvesting stage
T_1	532.49	548.16	522.03	136.88	196.17	199.99
T_2	518.38	542.51	517.46	227.94	249.16	249.79
T_3	516.29	540.59	500.94	200.25	240.52	244.88
T_4	506.84	523.91	494.34	162.27	195.60	239.45
T_5	518.02	542.84	508.33	177.09	189.72	185.93
T_6	506.85	537.07	496.00	151.58	160.55	159.36
T_7	498.77	495.18	476.66	149.01	152.05	149.57
T_8	523.77	518.09	508.41	136.03	150.41	146.37
S.E m	3.43	2.57	2.36	4.04	0.84	2.26
LSD (P=0.05)	9.85	7.37	6.76	11.61	2.42	6.49
C.V (%)	1.15	0.84	0.81	4.18	0.76	1.99

Proliferation of microorganism:

Due to the application of Zn fertilizer in different doses, in different stages of rice plant and by different application method has significantly stimulated the growth and microbial population of microorganisms (Total bacteria, actinomycetes and fungi) in soil (Table 5).Observation on bacteria, actinomycetes, and fungi were recorded at maximum tillering, panicle initiation and harvesting stage of rice plants and were statistically analysed.

Bacteria:

From the present experiment, it has been found that proliferation of bacteria was significantly increasing in all the treatments as compared to untreated control. As compare to the untreated control soil, the maximum stimulation of total bacteria was recorded in T7 treatment, when Zn was applied both as soil application and as foliar spray during panicle initiation stage. The least stimulation was observed when the fertilizer was applied as foliar spray only. From the above data table 7, it has been found that the stimulation of total bacteria was increased in panicle initiation than maximum tillering stage, but decrease in harvesting stage. The mean value of bacterial population data were graphically depicted in the Table 5.

This result indicated that plant was able to produce higher root exudates when zinc was applied as both foliar and in soil which had stimulatory effect on bacterial population. As exudates possesses large range of organic and inorganic substances secreted by roots into the soil, which inevitably leads to changes in its biochemical and physical properties[7].

Actinomycetes:

As compared to the untreated control soil, the maximum stimulation of actinomycetes was significantly recorded in T7 treatment, when Zn was applied both as soil application and as foliar spray during panicle initiation stage. But the least stimulation of actinomycetes was recorded when the fertilizer was applied as foliar spray only. It was also observed that, actinomycetes population was increased during panicle initiation stage than maximum tillering stage, but again decreased during harvesting stage. The mean value of bacterial population data were graphically depicted in the Table 5.

This result indicates that plant was able to produce higher root exudates when Zinc was applied as both foliar and in soil which turn had stimulatory effect on actinomycetes population.

Fungi:

As compared to untreated control soil, the maximum stimulation of total fungi was significantly recorded in T7 treatment, when Zn was applied both as soil application and as foliar spray during panicle initiation stage. And the least stimulation of fungi was recorded when fertilizer was applied as foliar spray only. Although stimulation of fungi population was increased as compared to untreated control when Zn was applied as foliar spray only (Table 5).

This result indicated that plant was able to produce higher root exudates when zinc was applied as both foliar and in soil which turn had stimulatory effect on fungi population.

Also, for bacteria, fungi, or actinomycetes, a common observation was that the highest proliferation occurred at panicle initiation stage irrespective of the kind of treatments done. This can be attributed to the fact that large amount of exudates are secreted by plants during panicle initiation stage.This is similar to the findings of Aul[1,2 and 4]who stated that exudates rates were, in general, lowest at seeding stage, increased until flowering but decreased at maturity.

Table 5.Microbial population count of zinc fertilised soil (cfu× 10^5 g^{-1})

Treatment	Bacteria			Actinomycetes			Fungi		
	Maximum tillering stage	Panicle initiation stage	Harvesting stage	Maximum tillering stage	Panicle initiation stage	Harvesting stage	Maximum tillering stage	Panicle initiation stage	Harvesting stage
T_1	171.77	202.69	114.00	159.18	187.78	101.71	26.12	52.92	30.46
T_2	230.11	296.80	143.56	210.19	276.08	130.49	38.72	68.13	43.80
T_3	235.95	295.36	171.52	236.95	294.25	160.36	46.37	77.04	51.54
T_4	242.71	318.74	171.10	257.74	306.83	169.73	66.15	83.43	68.80
T_5	263.10	328.41	200.26	257.80	321.45	189.67	84.23	105.41	70.13
T_6	294.04	372.79	236.51	292.50	344.56	208.34	107.39	128.23	90.23
T_7	297.29	399.91	253.74	296.20	368.23	222.38	113.00	154.13	119.98
T_8	209.93	251.53	138.58	196.80	204.08	126.04	31.89	66.34	49.25
S.E m	243.11	308.28	178.66	238.42	287.91	163.59	64.23	91.95	65.52
LSD (P=0.05)	1.75	2.87	2.27	2.86	2.35	2.04	1.44	2.34	1.49
C.V (%)	5.02	8.24	6.50	8.22	6.75	5.84	4.12	6.72	4.27

Dehydrogenase enzyme activity in soil

As compare to untreated control, from the present investigation, it was found that, all the Zn fertilizer treated treatments increase the dehydrogenase activity significantly during all the three stages of growth of rice plant. Among the treated treatment, maximum significant increase of dehydrogenase activity was recorded in T7 treatment, when Zn was applied both as soil application and as foliar spray during panicle initiation stage as compared to untreated control. But the least increase in dehydrogenase activity was recorded in T2 treatment when Zn was applied in soil @ 2.5 kg ha^{-1}during maximum tillering stage. The graphical representation of overall increase in dehydrogenase activity was shown in fig 1. This can be attributed the fact that application of $ZnSO_4.7H_2O$ had stimulatory effect on microorganisms which resulted in proliferation of soil microorganisms which ultimately resulted in significant increase in dehydrogenase activity. As dehydrogenase is an enzyme that occurs in all viable microbial cells, these enzymes function as a measurement of the metabolic state of soil microorganisms (5). Also dehydrogenase activity is one of the most adequate, important and one of the most sensitive bio indicators, relating to soil fertility [6 and 7].

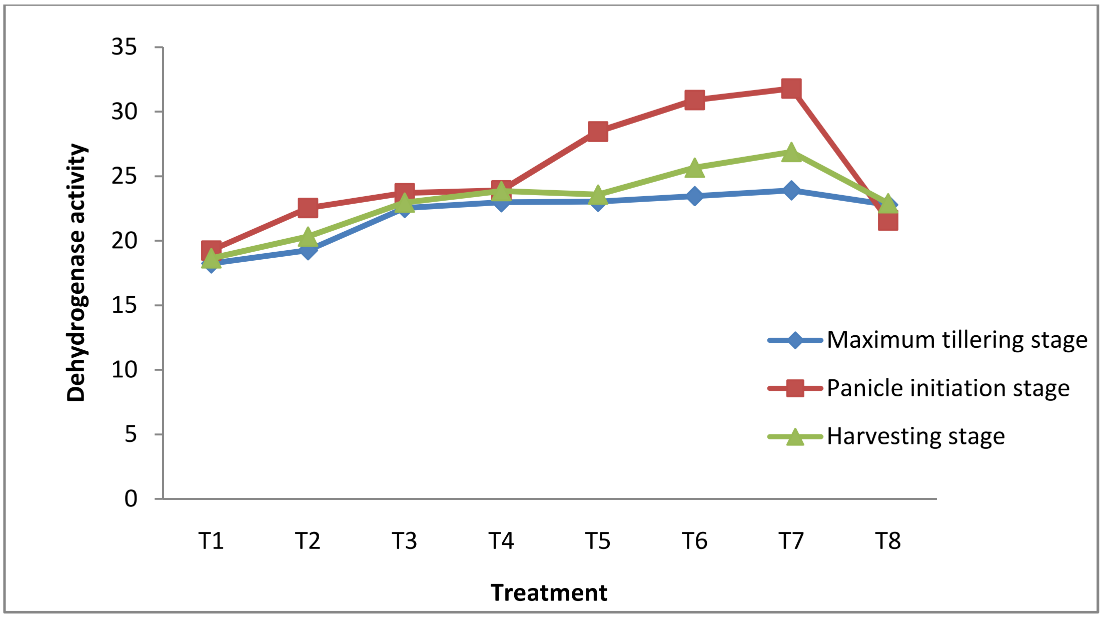

Fig 1: Effect of Zn on dehydrogenase enzyme [Amt of TPF formed (μg g^{-1} hr^{-1})]

Microbial biomass carbon of soil

Microbial biomass is a useful indicator of soil quality. Soil microorganisms such as bacteria, actinomycetes, fungi, are involved in several processes that influence soil quality and assessing the effect of management on soil microorganisms. Increases in microbial biomass over time are considered beneficial. They may indicate an increase in beneficial biological functions in soil and a future increase in organic carbon in soil. In contrast, a decline over time is considered to have a negative effect on soil quality.

Application of $ZnSO_4.7H_2O$ at different application doses, in different growth stages of rice plant, and by different application methods resulted in significant increase in microbial biomass carbon content for all treatments, over untreated control. It was observed that the content of microbial carbon was highly related to the mode of application of $ZnSO_4.7H_2O$ to the soil. From the present experiment, it was observed that the significant increase in microbial biomass in soil was highest in T7 treatment during panicle initiation stage, when Zn was applied both in soil application and as foliar spray as compare to untreated control. On the other hand, the increase in microbial biomass carbon was lowest in T2 treatment, during maximum tillering stage as compared to untreated control. But when plant reach to the maturity, then total microbial biomass again fall down (Table 6).

The increase in microbial biomass carbon can be attributed to the fact that incorporation of zinc resulted in increase in the proliferation of microbial population in soil system which ultimately resulted in increase in microbial biomass carbon content in soil. This sustained the finding of earlier worker (7 & 8) who reported that there was a positive correlation between microbial biomass carbon and the proliferation of soil microorganisms in the rice field. From this experiment it was revealed that application of Zinc at different rates at different mode of application highly induced the activities of soil microorganisms in the rice field.

Table 6: Effect of zinc on microbial biomass carbon of soil (mg/kg of dry soil)

Treatment	Maximum tillering stage	Panicle initiation stage	Harvesting stage
T_1	63.54	64.05	62.05
T_2	65.26	74.75	69.07
T_3	74.62	76.63	76.30
T_4	105.49	76.78	76.73
T_5	105.70	122.08	97.58
T_6	115.05	123.37	110.43
T_7	132.57	150.30	112.23
T_8	102.78	140.09	99.11
S.E m	1.81	3.58	3.16
LSD (P=0.05)	5.20	10.26	9.08
C.V (%)	3.28	5.98	6.23

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