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Impact of zinc and biofertilizers on nitrogen use efficiency in summer rice in acidic *terai* soil of West Bengal

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ABSTRACT

A field experiment was conducted for two years in 2018-19 and 2019-20 in acidic soil (pH 5.43) of the terai region of West Bengal to assess the effect of zinc (Zn) and biofertilizers (ZSB and VAM) on the nitrogen (N) uptake and use efficiency by summer rice. The soil of the study area was sandy loam, medium in available Zn (0.81 mg kg^{-1}) content and deficient in available N (173.11 kg ha^{-1}). The experiment was laid out in a randomized complete block design with fourteen treatment combinations including three N doses (80%, 100% and 120% of recommended dose), three Zn doses (25, 37.5 and 50 kg ha^{-1} $ZnSO_4,7H_2O$), two biofertilizers (ZSB and VAM) and one additional foliar Zn spray. Significantly higher uptake of N at different growth stages was noticed with the increasing N and Zn application. Combined use of ZSB and VAM was most effective for grain yield (5147.35 kg ha^{-1}) and N uptake by grain (65.55 kg ha^{-1}) and straw (40.11 kg ha^{-1}). Increasing N dose to 120% and Zn to 50 kg ha^{-1} without biofertilizers were not found effective to increase yield, N uptake and N use efficiency.

Keywords**:** Nitrogen uptake, Nitrogen use efficiency, Zinc, ZSB, VAM

INTRODUCTION

Rice is the most important staple food in India and provides 43% of the calorie intake of 70% population (Choudhary and Suri, 2018). Demographic studies have indicated that India requires about 130 mt of rice by 2025 with a yearly average hike of 3 mt to ensure national food and nutritional security vis-à-vis achieving the United Nations' Sustainable Development Goals (SDGs). The rice production in the conventional rice-wheat production system is facing an acute decline in factor productivity and yield stagnation due to nutritional deficiency and poor soil health (Singh *et al.,* 2021). Among the essential plant nutrients, nitrogen (N) is the most important factor for declining yield due to its inherent deficiency in soil and low use efficiency even if applied as fertilizers. Nitrogen use efficiency remained below 40% in cereals (Omara *et al.,* 2019) in intensive agricultural systems and the rest is lost through various processes such as leaching, denitrification, surface run-off, volatilization, and microbial assimilation (Banik and Mukhopadhyay, 2021). Several methods have been adopted for increasing N use efficiency by the crops. Among them, the use of microbial inoculation in conventional puddled rice has been reported to increase N use efficiency (Sahane *et al.,* 2019). After nitrogen, zinc (Zn) is the second most yield-limiting nutrient in rice (Quijano-Guerta *et al.,* 2002). Zn deficiency in rice reduces chlorophyll content in leaves and thereby decreases the photosynthesis rate. A deficiency of Zn lowers flowering and fruit development, and prolongs the growth period which eventually delays maturity, and reduces yield and grain quality. Sub-optimal nutrient use efficiency is also endorsed by Zn deficiency. Increasing the use efficiency of both N and Zn is imperative for rice productivity in all types of soils. Several studies have shown that the combined application of N and Zn either in soil or as foliar efficiently increased agronomic growth parameters and yield of rice and also grain Zn accumulation (Singh *et al.,* 2018; Wang *et al.,* 2022). The N reportedly has a positive role in increasing Zn content in grain by promoting root-to-shoot translocation of Zn (Ji *et al.,* 2022). On the other hand, Zn helps in N metabolism by promoting amino acids (lysine and tryptophan) and nucleic acid formation. Zn is essential for the activity of a large number of enzymes required for protein formation (Jarallah and Al-Amedi, 2017) and thus increases the demand for N by crops.

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This positive interaction between N and Zn had attained considerable attention in recent times to increase yield and grain Zn concentration to fight against rice yield loss and micronutrient malnutrition among the people. But the main focus of attention has been paid to increasing Zn use efficiency by applying N. However, limited information is available concerning how Zn application either as basal or foliar spray affects N uptake and use efficiency in rice in conventional practices in presence or absence of biofertilizers. Considering this the present study was undertaken to ascertain the effect of biofertilizers and graded doses of Zn on N uptake and use efficiency by rice in conventional practice in an acidic soil of terai region of West Bengal that is frequently reported as deficient in both N and Zn.

MATERIALS AND METHODS

The field experiment was conducted Central Research Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India (26°23'59.62" N and 89°23'23.90" E, altitude of 51m AMSL), in *boro* (summer) seasons of 2018-2019 and 2019-2020. The soil of the experimental field belongs to Typic Fluvaquents with 52.5, 34.9, and 12.6% of sand, silt and clay respectively, having sandy loam texture. The initial soil (0-0.15 m) was acidic (pH 5.43). The organic carbon (OC) content was 10.18g kg^{-1}. The available N, P_2O_5 and K_2O content were 173.11, 24.80 and 137.67 kg ha^{-1}, respectively. The DTPA-extractable Zn content was recorded as 0.81 mg kg^{-1}. The experiment was conducted in randomized complete block design with 14 treatments and three replications growing a medium duration (120-130 days) rice cultivar, MTU 1153 (*Chandra*). The NPK and Zn recommendation (RDF) for summer rice in this region is 100:50:50 kg ha^{-1}, and 25 kg $ZnSO_4$ ha^{-1}. The treatment combinations were: T_1- 0% N + 0% Zn (control), T_2- 80% N + 25 kg ha^{-1} $ZnSO_4$, T_3- 80% N + 37.5 kg ha^{-1} $ZnSO_4$, T_4- 80% N + 50 kg ha^{-1} $ZnSO_4$, T_5-100% N + 25 kg ha^{-1} $ZnSO_4$, T^6- 100% N + 37.5 kg ha^{-1} $ZnSO_4$, T_7- 100% N + 50 kg ha^{-1} $ZnSO_4$, T_8-100% N + 25 kg ha^{-1} $ZnSO_4$ + ZSB, T_9-100% N + 25 kg ha^{-1} $ZnSO_4$ + VAM, T_{10}-100% N + 25 kg ha^{-1} $ZnSO_4$+ZSB+VAM, T_{11}-100% N+ 25 kg ha^{-1} $ZnSO_4$ + foliar spray of Zn at maximum tillering and booting stage, T_{12}-120%N+25 kgha^{-1} $ZnSO_4$, T_{13}- 120% N + 37.5 kg ha^{-1} $ZnSO_4$ and T_{14}-120% N+50 kg ha^{-1} $ZnSO_4$. The zinc solubilizing bacteria (ZSB) was applied as root dipping before transplanting in an aqueous solution. The Vesicular Arbuscular Mycorrhiza (VAM) was mixed with well-decomposed vermicompost and applied to seedling roots. Rice seedlings were manually transplanted in the main experimental plot as two seedlings per hill with 20 × 15 cm hill spacing in 4 × 4 m^2 plots. Standard agronomic management practice was followed to grow the crop. Recommended doses of P_2O_5 and K_2O were applied in the soil at the final land preparation in the forms of single super phosphate (SSP) and muriate of potash (MoP). Nitrogen was applied as urea in three split doses each at transplanting, maximum tillering and booting stages. The foliar spray of Zn was applied as 0.5% $ZnSO_4$ aqueous solution.

The rice grain yield was calculated from a harvest area of 4 m^2 quadrate from the central position in each plot and adjusted to 14% moisture content. Nitrogen content in the parts at different growth stages was measured by the modified Kjeldhal method as described by Jackson (1967) after digesting the samples in H2SO4 at a temperature 370^{0}C. The N uptake was computed by multiplying N content with dry matter production accordingly in different growth stages.

CALCULATION OF N USE EFFICIENCIES

Nitrogen use efficacy by rice was quantified by calculating various indices, namely partial factor productivity of N (PFP_N), agronomic nitrogen use efficiency (ANU), Apparent nitrogen recovery (ANR), Grain N harvest index (Grain N HI), and nitrogen physiological efficiency index (NPEI) were calculated using the equations given below (Dobermann 2005):

$$PFP_N = Yt / Na$$

$$ANU = (Yt - Yc) / Na$$

$$ANR = (U_N - Uc) / Na$$

$$Grain\ N\ HI = U_{N\ (Grain)} / U_{N\ (Grain + Straw)}$$

$$NPEI = Yt / U_{N\ (Grain + Straw)}$$

Wherein, Yt and Na refer to grain yield (kg ha^{-1}) and N applied (kg ha^{-1}); Yc is the grain yield (kg ha^{-1}) in control plot where no N was applied; U_N and U_C refer to total N uptake (kg ha^{-1}) in fertilized plot and control plot, respectively; $U_{N\ (Grain)}$ and $U_{N\ (Grain + Straw)}$ represents N uptake (kg ha^{-1}) in grain and grain+straw, respectively.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) in RCBD was performed using IBM SPSS (version 26.0, SPSS Inc. Chicago, IL, USA) for pooled data from two years of field experiments. The significance of the difference between means was determined at 5% ($P<0.05$) level of probability using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Nitrogen uptake at different growth stages

Observation of data presented in Table 1 reveals that N uptake by rice plants increased progressively in all growth stages with the increased doses of N irrespective of Zn doses in both years. The analysis of pooled data for these consecutive two years experiments showed that, N uptake by the plant significantly (at $p \leq 0.05$) increased from 26.32 kg ha^{-1} in T1 (0% N + 0 Zn) (control) to 55.33 kg ha^{-1} in T10 (100% N+25 kg ha^{-1} $ZnSO_4$ + VAM + ZSB) in the tillering stage. However, the application of Zn in higher doses further increased N uptake in the corresponding N application rate. Nitrogen uptake in tillering stage significantly increased from 33.15 kg ha^{-1} to 41.04 and 48.28 kg ha^{-1} when N application was increased from 80% to 100 and 120% of RDF, respectively with 25 kg ha^{-1} $ZnSO_4$, while it was 38.85, 45.30 and 53.08 kg ha^{-1} when Zn was applied at 50 kg ha^{-1}. The effect of individual application of ZSB (T8) and VAM (T9) was not statically much different than 100% N application with all doses of Zn from 25 to 50 kg ha^{-1}, but the combined application of ZSB and VAM substantially increased N uptake. Foliar application of Zn with 100% N and 25 kg ha^{-1} basal Zn did not show any significant effect on N uptake even though additional foliar application increased N uptake with the same rate of soil Zn application. Higher rates of soil Zn application (37.5 and 50 kg ha^{-1}) were found more effective to increase N uptake than combining an additional foliar Zn application with 100% N and 25 kg ha^{-1} $ZnSO_4$ in both years. Obviously, N application at 120% of RDF significantly (at $p \leq 0.05$) increased N uptake by rice plants even more than the application of ZSB and VAM separately with 100% N. Response of N and Zn application with or without ZSB and VAM in panicle initiation and flowering stages was similar to tillering stage except the higher N uptake in later growth periods due to the gradual accumulation of N in plant parts.

Table 1: Effect of treatments on N uptake by rice in different vegetative growth periods (on pooled data of two years experiments with three replications for each treatment)

Treatments	Tillering stage			Panicle Initiation Stage			Flowering Stage		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
T^1	22.85a	29.78a	26.32a	33.18a	41.33a	37.25a	48.48a	54.49a	51.49a
T^2	29.19b	37.11b	33.15b	41.33b	48.15ab	44.74b	57.81b	63.18b	60.50b
T^3	32.53bc	39.44bc	35.99bc	44.14bc	52.44bc	48.29bc	59.29b	66.11b	62.70bc
T_4	34.95bcd	42.75bcd	38.85bcd	47.61bcd	54.79bcd	51.20cd	62.15bc	69.26bc	65.71cd
T_5	37.21bcde	44.87cde	41.04cd	50.55cde	57.82cde	54.19de	65.23cd	73.41cd	69.32de
T_6	38.81cde	47.10cde	42.96cd	53.15cdef	59.67cde	56.41def	67.81d	75.85cde	71.83ef
T_7	41.16cdef	49.43def	45.30def	55.53defg	63.44efg	59.49efg	70.15de	78.52def	74.34fg
T_8	37.71bcde	46.78cde	42.25cde	52.17cde	59.34cde	55.75def	67.37cd	74.67cde	71.02ef
T_9	39.27cde	48.15def	43.71def	54.38defg	61.18def	57.79ef	68.45d	78.18def	73.32ef
T_{10}	50.48g	60.18g	55.33h	65.47h	72.43h	68.95i	81.80g	89.50h	85.65j
T_{11}	38.11bcde	45.89cde	42.00de	51.48cde	58.52cde	55.01de	66.47cd	73.91cd	70.19ef
T_{12}	43.78defg	52.77efg	48.28efg	58.42efgh	65.04efgh	61.73fgh	73.78e	81.47efg	77.62gh
T_{13}	45.51efg	55.46fg	50.49fgh	61.52fgh	68.81fgh	65.17ghi	75.11ef	83.53fgh	79.32hi
T_{14}	48.33fg	57.82g	53.08gh	62.85gh	70.09gh	66.47hi	79.67fg	86.81gh	83.24ij
SEM (±)	2.74	2.42	2.18	2.79	2.58	1.91	1.67	2.25	1.36
LSD(P=0.05)	7.96	7.04	6.34	8.10	7.50	5.54	4.84	6.54	3.95

Different letters in the same column are significantly different by Duncan Multiple Range Test (DMRT) at p≤05

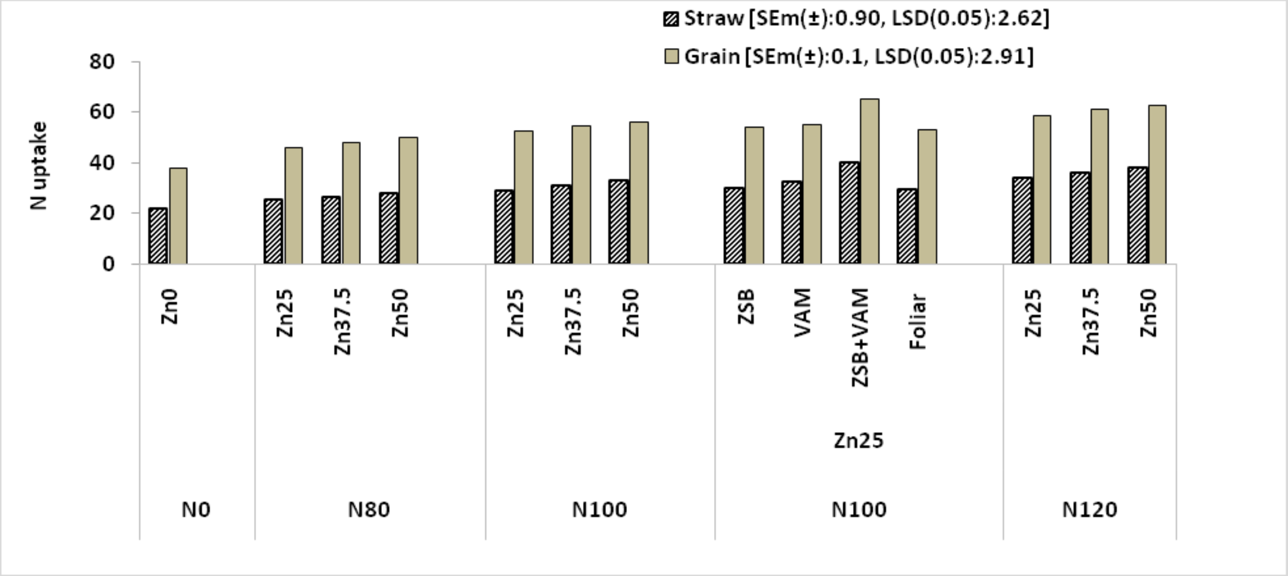

Fig. 1: Effect of N, Zn and biofertlizers on nitrogen uptake (kg ha^{-1}) by rice straw and grain at harvest (on pooled data)

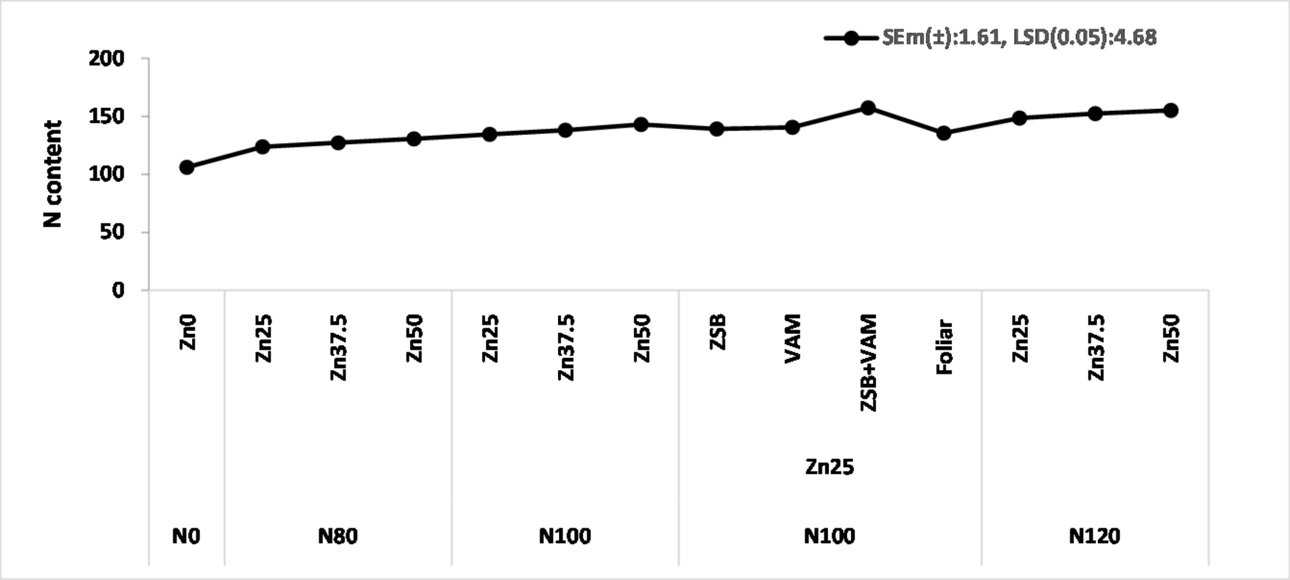

Fig. 2: Available nitrogen (kg ha^{-1}) in soil at harvest of two years experiment

The higher concentration of N at the advanced growth stages is mainly driven by the increase in total dry matter production by rice. Sahane *et al*. (2019) noted the role of increasing dry matter in improving N uptake toward crop maturity. Such a positive effect of Zn application on N uptake was also reported by Yang *et al*. (2021) and Tuiwong *et al*. (2022). Applying Zn to rice significantly increases the photosynthetic period, better root and shoot growth, and yield. Higher N acquisition by plants is essential for such growth resulting in higher N uptake at different growth stages.

Nitrogen uptake at the harvesting stage by grain and straw is presented in Figure 1. The application of increasing doses of N and Zn increased N uptake by rice straw and grain. The highest N uptake by both grains and straw was noted in T10 (100% N+25 kg ha^{-1} $ZnSO_4$ + VAM + ZSB). The uptake of grains was more than that of straw in all treatment combinations because of the remobilization of N from senescing leaves and stems to the grains during the reproductive stage (Lemaître *et al.,* 2008). Nitrogen, being an essential component of proteins, tends to accumulate in the grains. During the vegetative stage N is taken by the plants and is used to produce new leaves, stems and roots. During the reproductive stage, most of the uptaken N moves towards grains. It is reported that Arbuscular Mycorrhizal Fungi like VAM enhance the concentration of nutrients including N significantly leading to increase photosynthate production and hence biomass accumulation in different types of plants (Mitra *et al.,* 2019). The solubilization of native and applied Zn in the soil is accelerated by ZSB, especially in acidic soil by the hydrolysis of zinc phosphates and zinc carbonates (Upadhayay *et al.,* 2022) and thus helps to increase the positive interaction between N and Zn. Increasing N and Zn application rates also increases the available N content in soil at harvest (Figure 2). Incorporation ZSB and VAM were found very effective to increase N availability in the soil even in lower rates of N and Zn application.

Table 2: Effect of nitrogen, Zn and biofertilizers on yield and nitrogen use efficiency in rice (on pooled data of two years)

Treatments	Grain yield (kg/ha)	PFP_N*	ANU	ANR	Grain N HI	NPEI
T^1	3064.79 a	-	-	-	63.83	51.20 bcd
T^2	3790.06 b	47.38 g	9.07 a	0.096 a	64.62	53.40 de
T^3	3982.96 c	49.79 h	11.48 c	0.123 ab	64.45	53.41 cde
T_4	4184.14 e	52.30 j	13.99 f	0.146 bc	64.35	53.94 de
T_5	4079.03 d	40.79 c	10.14 b	0.144 bc	64.61	50.07 abc
T_6	4290.10 f	42.90 d	12.25 d	0.164 bcd	64.02	50.29 abcd
T_7	4514.65 h	45.15 e	14.50 g	0.181 cde	63.13	50.59 abcd
T_8	4621.24 ij	46.21 f	15.56 i	0.158 bcd	64.22	54.95 e
T_9	4584.93 i	45.85 f	15.20 hi	0.172 cde	63.19	52.30 cde
T_{10}	5147.35 m	51.47 i	20.83 k	0.273 f	62.04	48.72 ab
T_{11}	4970.80 l	49.71 h	19.06 j	0.151 bc	64.60	60.20 f
T_{12}	4426.26 g	36.89 a	11.35 c	0.170 cde	63.33	47.79 a
T_{13}	4658.08 j	38.82 b	13.28 e	0.191 de	63.02	47.99 a
T_{14}	4867.71 k	40.56 c	15.02 h	0.205 e	62.48	48.39 ab
SEM (±)	16.25	0.17	0.17	0.012	0.93	1.03
LSD(P=0.05)	47.25	0.49	0.50	0.034	NS	3.00

Different letters in the same column are significantly different by Duncan Multiple Range Test (DMRT) at p≤05

**PFP_N: Partial factor productivity of nitrogen; ANU: Agronomic nitrogen use efficiency; ANR: Apparent nitrogen recovery; Grain N HI: Grain nitrogen harvest index; NPEI: Nitrogen physiological efficiency index*

Grain Yield and nitrogen use efficiency

The data on grain yield and N use efficacies are presented in Table 2. The yield of rice grain increased with increasing doses of N and Zn. The highest yield was recorded in T10 (100% N+25 kg ha^{-1} $ZnSO_4$ + VAM + ZSB) which was immediately followed by T11 (100% N+25 kg ha^{-1} $ZnSO_4$ + Foliar Zn spray). The PFP_N was significantly (at $p \leq 0.05$) highest in T4 (80% N + 50 kg ha^{-1} $ZnSO_4$) and lowest in T12 (120% N + 25 kg ha^{-1} $ZnSO_4$). The higher value of PFP_N indicates a smaller amount of N fertilization is required to produce the expected yield (Shivay and Prasad, 2012). Thus, a higher dose of N application beyond recommended dose showed a lower PFP_N value. Significantly (at $p \leq 0.05$) highest ANU and ANR values were recorded in T10 showing the highest utilization of N by the application of combined doses of ZSB and VAM. However, lower values of ANU and ANR at the highest N application rate (120% of RDF) suggest the lower utilization of excess applied N that may be due to the various losses of N in soil. The Grain N HI showed insignificant variations in different treatments. The NPEI was significantly varied with increasing doses of N, but less effect was noticed by increasing Zn application in each N dose.

CONCLUSION

The results obtained in this two years field experiment showed that the uptake of N by rice in different growth periods increases with increasing N application rate. Zinc act in a synergistic way to transport N from soil to grain. Application of higher doses of both N and Zn beyond the recommended dose was not supportive for increasing N use efficiency in rice, rather the excess N may be lost through various possible mechanisms. According to the results, combined use of biofertilizers like VAM and ZSB with the recommended dose of N and Zn is suggested to increase yield, N uptake, and use efficiency in rice in this terai soil.

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