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Role of zinc, nitrogen and phosphorus fertilization, and use of microbial inoculation in zinc nutrition of wheat (*Triticum aestivum*) under different crop establishment methods

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ABSTRACT

A field experiment was conducted during *rabi* season of 2013-14 and 2014-15 at the Research Farm of ICAR-Indian Agricultural Research Institute, New Delhi to study the role of N, P and Zn fertilization, and microbial inoculation on concentration and uptake of zinc (Zn) in wheat plant ('HD 2967') at different growth stages, and DTPA-extractable Zn content in soil under three different crop establishment methods (CEMs), viz. conventional drill-sown wheat (CDW), system of wheat intensification (SWI) and zero tillage wheat (ZTW). Experiment was conducted in split-plot design with three CEMs as main plot, viz. CDW, SWI and ZTW. In subplot, two rate of N and P application [100% recommended dose of nutrients ($N_{120}P_{25.8}$) (RDN) and 75% RDN] and two microbial inoculations (MI), viz. *Anabaena* sp. (CR1) + *Providencia* sp. (PR3) (MI1) and *Anabaena-Pseudomonas* biofilmed formulation (MI2) was applied with 75% RDN making total four combination which were applied with and without Zn (5 kg Zn/ha through zinc sulphate heptahydrate) along with absolute control. Zinc fertilization increased Zn concentration in wheat grain by 3 and 2.9-3.2 mg/kg when Zn was applied with 100% RDN and 75% RDN + MI1 and 75% RDN + MI2, respectively which showed the role of Zn fertilization in Zn nutrition of wheat. Application of 100% RDN increased total Zn uptake by 78 and 180 g/ha over 75% RDN and control in Zn applied treatments which showed the role of N and P application on Zn nutrition of wheat. Application of MI1 and MI2 with 75% RDN increased grain Zn concentration by 2.3 and 2.5 mg/kg indicating their role in Zn nutrition of wheat. Among CEMs, ZTW was found superior and increased Zn concentration in wheat grain by 4.7 and 4.5 mg/kg over CDW and SWI, respectively. Soil DTPA-extractable Zn content was increased in all Zn applied treatments at harvest over initial level which indicates the positive effect of Zn fertilization on soil Zn status. Our study showed that application of recommended dose of Zn alone was not good enough to have proper Zn nutrition of wheat and to increase concentration and uptake of Zn in wheat plant and grain, but application of N and P at recommended rate and selecting suitable CEM is equally important.

Key words: *Anabaena-Pseudomonas* biofilmed formulation, DTPA-extractable zinc, System of wheat intensification, Zero tillage wheat, Zinc fertilization

Wheat (*Triticum aestivum* L.) is the crop which has served and still serving as a staple food of world and passed successfully through wheat revolution called as 'Green Revolution' in India to justify its status as staple crop grown on 30.23 million ha area with production of 93.5 million tonnes (Anonymous 2016). Being a staple food crop, slight variation in nutritional quality of wheat is going to affect nutritional status of diet of large section of people in India and world. Among the major nutritional indicators, zinc (Zn) nutrition of wheat was selected for study in two years field experiment. Justification of selecting Zn nutrition of wheat in present study was based on its deficiency in soil, plant and human being. Tandon (2013) reported that 49% of Indian soils are deficient in Zn; while Shivay *et al.* (2008a) mentioned yield improvement of wheat. Kumar *et al.* (2015) observed the increase in Zn concentration in wheat grain due to Zn fertilization. In regarding to Zn deficiency in human being, Palmgren *et al.* (2008) correctly reported that, among the major health related risk in Asia and world as a whole, Zn deficiency ranks 5th and 11th, respectively. Role of Zn fertilization in improving yield and its concentration in wheat grain changes with variation due to crop establishment methods (CEMs) and application rate and sources of major nutrients even at same rate Zn application, which need to study to understand the complete effect of Zn fertilization on Zn nutrition of wheat. Rate and sources of application of nitrogen (N) and phosphorus (P) creates variation in growth and yielding ability of plants which results in to variation in other nutrient uptake from soil. Improvement

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in Zn concentration in wheat grain when applied with N over only Zn application was cited in Wang *et al.* (2017).

Along with nutrient managements, role of crop establishment methods on Zn nutrition is need to be investigated as crop establishment methods also influence the growth and nutrient content in wheat. With increasing awareness about bio-fertilizers and its role in fixing atmospheric N (Kaushik 2014) and solubilising P (Dwivedi *et al.* 2004), its application was widely recommended to reduce cost on purchase of chemical fertilizer. In this regard it is worthwhile to evaluate the effect of combined application of microbial inoculation with chemical fertilizer on Zn nutrition of wheat. With this background, a field experiment was planned to study the role of N and P fertilizer, microbial inoculation and Zn fertilization on Zn concentration and uptake in wheat and variation in soil DTPA-extractable Zn content in soil at different growth stages.

MATERIALS AND METHODS

A field experiment was conducted in winter (*rabi)* season for two consecutive years (2013-14 and 2014-15) at the Research Farm of ICAR-Indian Agricultural Research Institute, New Delhi located at the latitude of 28°38' N, longitude of 77°10' E and altitude of 228.6 m above the mean sea level. The climate of Delhi is of sub-tropical and semi-arid type with hot and dry summer and cold winter and falls under the agro-climatic zone 'Trans-Gangetic plains'. The mean annual normal rainfall is 650 mm and annual mean pan evaporation is about 850 mm. The soil of experimental field was sandy clay loam in texture having *p*H 7.6, organic carbon (0.54%) and available N, P, K and DTPA-extractable Zn of 200.3 kg/ha, 23.3 kg/ha, 284.6 kg/ha and 0.87 mg/kg, respectively (Prasad *et al.* 2006).

The experiment involving wheat variety 'HD 2967' was planned in split-plot design comprising of three crop establishment methods (CEMs), viz. conventional drill-sown wheat (CDW), system of wheat intensification (SWI) and zero tillage wheat (ZTW) as main plots with nine nutrient management options as sub-plot treatments. Sub-plot treatments involved recommended dose ($N_{120}P_{25.8}$) (RDN), 75% RDN, 75% RDN + *Anabaena* sp. (CR1) + *Providencia* sp. (PR3) (MI1) and 75% RDN + *Anabaena-Pseudomonas* biofilmed formulation (MI2) with and without Zn which made total eight options along with one absolute control ($N_0P_0Zn_0$). In order to have same crop growth duration in all three CEMs, sowing was done on same date. In ZTW, direct sowing of 115 kg seed/ha was done with row spacing of 20 cm using seed-drill. In SWI, 1-2 seeds were dibbled per spot at a spacing of 20 cm × 20 cm. In CDW, sowing was done with seed drill using 100 kg seed/ha with a row spacing of 22.5 cm. For application of biofertilizer inoculants, a thick paste of respective culture was made in carboxy methyl cellulose (CMC) and seeds were treated with this thick paste and dried in shade for half an hour just before sowing. Nitrogen was applied in three equal splits i.e. 1/3 at sowing, 1/3 at 30 days after sowing (DAS) and 1/3 at 60 DAS. Potassium (K) was applied uniformly at the rate of 49.8 kg K/ha. In case of Zn, soil application of 5 kg Zn/ha through Zn sulphate heptahydrate was followed in both seasons of wheat crop at the time of sowing. Application of all doses of P, K and Zn was done at the time of sowing as per treatment details. For weed management, hand weeding was done twice in all CEMs. Irrigation was given as per standard recommended practices.

For determination of Zn from wheat plant, representative sample from each replication in all treatments were selected. These plants were then air dried and further dried in a hot air oven at temperature of 60±2^{0}C till constant weight was obtained. Dry weight was recorded and used for calculation of Zn uptake. After recording dry weight, same plant samples were used for Zn determination. Zinc was analyzed on a di-acid (perchloric acid ($HClO_4$) + nitric acid (HNO_3) in 3:10 ratio) digest on an atomic absorption spectrophotometer (Prasad *et al.* 2006). At harvest, grain and straw samples from each plot was taken for determination of Zn concentration as above-mentioned procedure. For determination of Zn uptake in grain and straw of wheat, the net plots were harvested and sun-dried in the field and then weighed to record the total biomass yield. Grain yield was measured after cleaning and drying. Weighing of the grain was done at 14% moisture content, while straw yield was calculated by subtracting grain yield from the total biomass yield.

For collection and preparation of representative soil sample for determination DTPA-extractable Zn content from soil sample, procedure given by Prasad (1998) was followed. Soil DTPA-extractable Zn content was determined by procedure of Lindsay and Norvell (1978). Dehydrogenase activity was assayed using soil incubated with triphenyl tetrazolium chloride (3%) for 24 hours in dark. Methanol was added to terminate the enzymatic reaction and the supernatant was filtered and absorbance taken at 485 nm (Casida *et al*., 1964). The values were expressed as μg of triphenyl formazon (TPF)/g/hr. All the data obtained from the experiment were statistically analysed using the F-test as per the standard statistical procedure (Gomez and Gomez, 1984) and least significant difference (LSD) values (P = 0.05) were used to determine the significance of difference between treatment means.

RESULTS AND DISCUSSION

Zinc concentration and uptake

Zinc concentration showed decreasing trend toward crop maturity with the highest value at 30 DAS and the lowest in wheat straw which arises out of dilution effect of increasing in dry matter of plant, decrease in absorption toward maturity and translocation of most of zinc from straw to wheat grain. Concentration in grain was higher by 1.72-1.91 times than straw (Table 1). Shivay *et al.* (2008a) also reported higher Zn concentration in wheat grain than wheat straw. In case of uptake, it showed increasing trend from 30 DAS with highest in grains. Increase in uptake was recorded even though concentration decreased as crop turned towards maturity and thus it indicated the

Table 1 Concentration and uptake of Zn as influenced by crop establishment methods, microbial inoculants and rate of nitrogen and phosphorus application (data pooled over two years)

Treatment	Concentration (mg/kg dry matter)					Uptake (g/ha)					
	30 DAS	60 DAS	90 DAS	Straw	Grain	30 DAS	60 DAS	90 DAS	Straw	Grain	Total
Crop establishment methods											
CDW	99.2	66.4	42.8	17.5	33.5	30.1	151.2	292.3	109.9	146.3	256.2
SWI	99.9	66.8	43.0	17.8	33.7	30.3	151.9	293.6	111.0	146.5	257.5
ZTW	101.7	71.3	47.3	22.2	38.2	31.2	165.6	330.0	146.8	175.4	322.2
SEm±	0.50	0.46	0.36	0.20	0.25	0.18	1.16	2.59	0.78	0.48	1.24
CD (P=0.05)	1.95	1.80	1.43	0.78	0.97	0.71	4.54	10.16	3.08	1.88	4.86
Nutrient management treatments											
Control ($N_0P_0Zn_0$)	90.2	54.3	31.5	11.6	23.4	25.0	107.7	190.0	65.2	87.6	152.8
RDN ($N_{120}P_{25.8}$)	101.5	69.7	45.9	20.1	36.4	31.3	161.3	320.8	129.6	163.7	293.3
RDN + Zn*	102.7	74.2	49.6	22.1	39.4	31.8	179.8	356.1	148.3	184.9	333.2
75% RDN ($N_{90}P_{19.35}$)	100.7	65.0	41.5	17.6	33.5	30.5	141.8	272.5	107.4	141.6	249.0
75% RDN + Zn	100.8	65.6	42.1	17.9	33.7	30.6	145.0	279.7	110.4	144.6	255.1
75% RDN + MI1	101.1	68.8	45.2	19.6	35.8	31.1	157.9	313.9	124.7	158.8	283.6
75% RDN + MI1+ Zn	101.9	73.2	48.7	21.7	38.7	31.5	175.9	346.8	144.4	180.0	324.4
75% RDN + MI2	101.3	69.2	45.5	19.8	36.0	31.2	159.1	316.7	126.6	160.7	287.3
75% RDN + MI2 + Zn	102.3	73.8	49.2	22.0	39.2	31.7	177.7	351.5	146.4	182.6	328.9
SEm±	0.78	0.73	0.57	0.32	0.38	0.28	1.74	3.97	1.54	1.24	2.71
CD (P=0.05)	2.22	2.06	1.62	0.91	1.09	0.78	4.96	11.28	4.38	3.53	7.71

MI1: *Anabaena* sp. (CR1) + *Providencia* sp. (PR3); MI2: *Anabaena-Pseudomonas* biofilmed formulation; Zn*: Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; RDN: Recommended dose of nutrients

dominant role of dry matter production over concentration in deciding Zn uptake. Uptake in grain was higher by 29-36 g/ha than wheat straw which could be attributed to higher concentration in grain, although straw yield was higher than grains yield. Crop establishment methods played a significant role in affecting Zn concentration in wheat and among the crop establishment methods ZTW was statistically superior to CDW at all stages and in grain and straw; while it had significantly higher concentration over SWI at 60 and 90 DAS and in grain and straw. Zero tillage wheat had 4.7 and 4.5 mg/kg higher Zn concentration in wheat grain than CDW and SWI, respectively which signifies the role of CEMs in Zn nutrition of wheat. Uptake of Zn was increased by 29.1 g/ha in wheat grains in ZTW over CDW; while increase over SWI was 28.9 g/ha. This higher uptake was contributed by both higher concentration and higher dry matter production in ZTW over CDW and SWI. Singh *et al.* (2017) also reported variation in concentration and uptake of nutrient among ZTW and CDW.

Role of Zn fertilization in improving Zn nutrition of wheat over inherent soil Zn supply was justified from significantly higher concentration of Zn in all Zn applied treatment over Zn omitted ones at all growth stages and in grains and straw (Table 1). Increase in Zn concentration due to Zn fertilization over Zn omitted control was the highest at 60 DAS; while Zn fertilization increased Zn concentration in wheat grain by 3.0, 0.2, 2.9 and 3.2 mg/kg when Zn was applied with 100% RDN, 75% RDN, 75% RDN + MI1 and 75% RDN + MI2, respectively. Treatment containing 100% RDN recorded significantly higher increase in grain Zn concentration over 75% RDN which showed the positive effect of application of recommended dose of nutrients (N and P) on Zn nutrition. Concentration of Zn in wheat grain with 75% RDN was significantly higher than control which signifies the role of major nutrient application in increasing Zn concentration in wheat. Zinc concentration in wheat grain with 100% RDN was increased by 2.9 and 13 mg/kg over 75% RDN and control. Treatment containing application of 75% RDN + MI1 and 75% RDN + MI2 showed an increase of 2.3 and 2.5 mg/kg in Zn concentration in wheat grain over 75% RDN in Zn omitted treatment. Similarly, increase was 5.0 and 5.5 mg/kg in respective treatments over 75% RDN in Zn applied treatments. This kind of behaviour is an indication of role played by application of microbial inoculation with 75% RDN in Zn nutrition of wheat. Rana *et al.* (2012) also mentioned the role of microbial inoculation in enhancing micronutrient concentration especially Zn in wheat grain.

Dry matter accumulation was affected by application of primary nutrients (N and P), microbial inoculation and Zn fertilization (Fig 1a). Role of application of primary nutrients is judged from significantly higher dry matter accumulation recorded with 100% RDN over 75% RDN and control and superiority of 75% RDN over control.

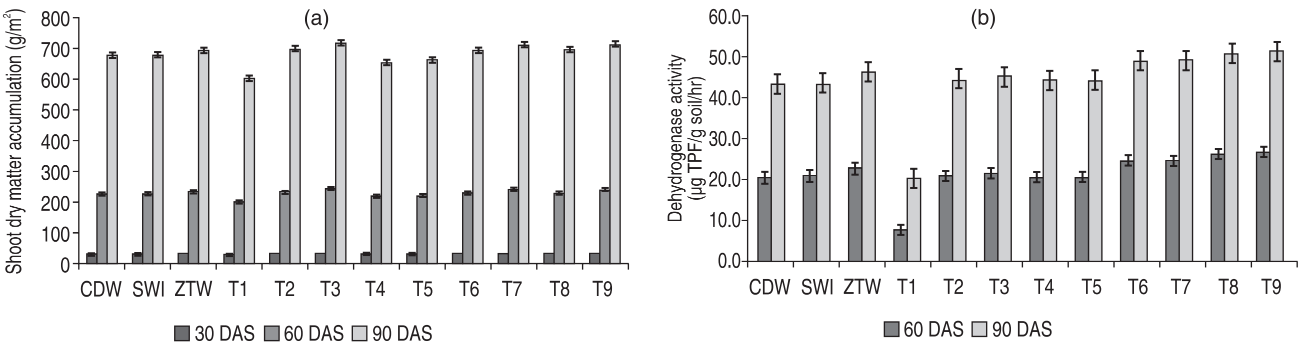

Fig 1 Effect of CEMs and nutrient management treatments on shoot dry matter accumulation (a) and dehydrogenase activity (b) (data pooled over two years). CDW: Conventional drill-sown wheat; SWI: System of wheat intensification; ZTW: Zero tillage wheat; T1: Control ($N_0P_0Zn_0$); T2: RDN ($N_{120}P_{25.8}$); T3: RDN + Zn; T4:75% RDN ($N_{90}P_{19.35}$); T5: 75% RDN + Zn; T6: 75% RDN + MI1; T7: 75% RDN + MI1 + Zn; T8: 75% RDN + MI2 and T9: 75% RDN + MI2 + Zn; RDN: Recommended done of nutrients.

Microbial inoculation contributed similar quantity of dry matter as that of 100% RDN; while the role of Zn fertilization was least and occurred when Zn was applied with 100% RDN or with 75% RDN + MI1 or MI2 and not when applied with 75% RDN. Positive effect of application of N and P on dry matter accumulation was also cited by Chaturvedi (2006) and Sharma *et al.* (2012), respectively; while Nain *et al.* (2010) mentioned the role of microbial inoculation in increasing dry matter accumulation. This variation in dry matter production played a significant role in differential Zn uptake. Uptake was significantly higher in all Zn applied treatments than Zn omitted treatments. In fact, concentration played a major role than dry matter production in Zn uptake as Zn application had least effect on dry matter production (Table 1). Application of 100% RDN and microbial inoculations also played significant role in increasing uptake of Zn. Total Zn uptake in 100% RDN was higher by 78.1 and 44.3 g/ha than 75% RDN with and without Zn application, respectively; while increase in uptake over control was 180.4 and 140.5 g/ha. Total uptake in 75% RDN + MI1 was increased by 69.3 and 34.6 g/ha with and without Zn application over 75% RDN; while increase in 75% RDN + MI2 over 75% RDN was 73.8 and 38.3 g/ha with and without Zn application. Role of Zn fertilization (Bharati *et al.* 2013) and application of N (Jarallah and Al-Amedy 2017) in increasing Zn uptake has also been reported earlier.

Dehydrogenase activity in soil

In order to distinguish the role of inherent microbial population and externally applied microbes through microbial inoculation, soil dehydrogenase activity was measured at 60 and 90 DAS in both years of experiment (Fig 1b). Dehydrogenase activity which was significantly higher in inoculated treatment than uninoculated treatment both at 60 and 90 DAS, it indicated that major part of microbial

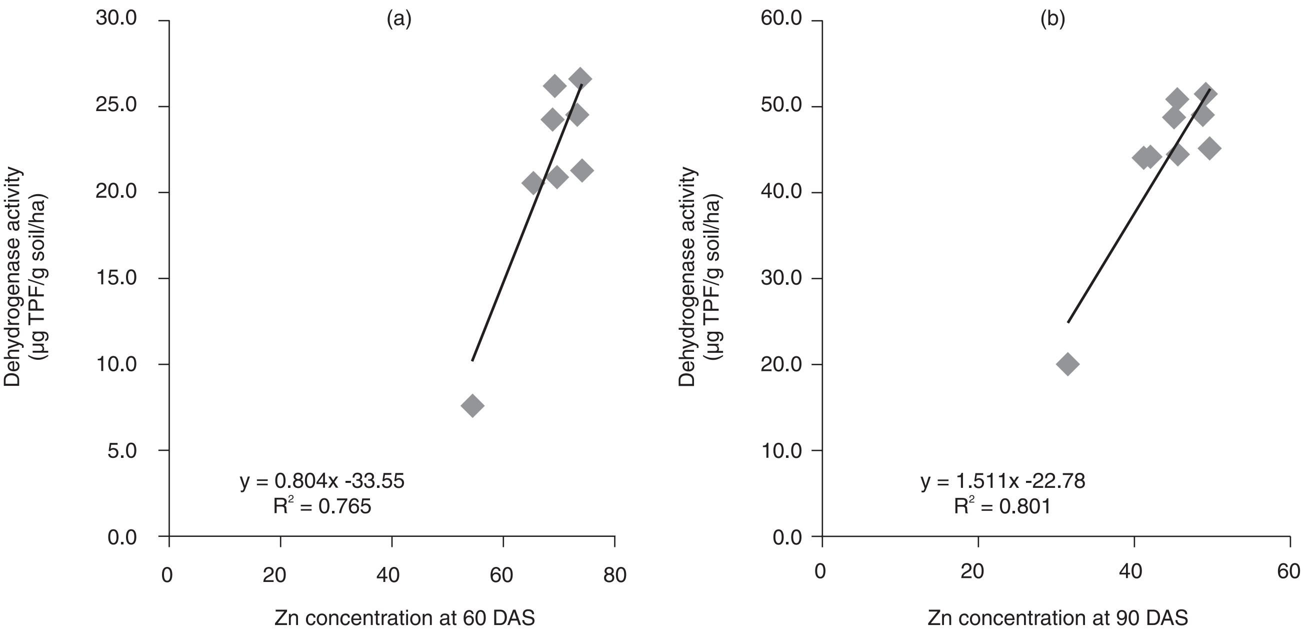

Fig 2 Correlation between Zn concentration and dehydrogenase activity in soil at 60 DAS (a) and 90 DAS (b) as influenced by nutrient management treatments (data pooled over two years).

Table 2 Effect of crop establishment methods and nutrient management options on DTPA-extractable zinc (g/ha) in wheat soil at different growth stages during 2013-14 and 2014-15

Treatment	2013-14					2014-15				
	Initial	30 DAS	60 DAS	90 DAS	At harvest	Initial	30 DAS	60 DAS	90 DAS	At harvest
Crop establishment methods										
CDW	2876.2	5128.0	4897.4	4099.8	3732.9	3046.4	5198.6	4879.0	4139.2	3773.9
SWI	2874.3	5125.5	4894.2	4097.0	3729.4	3044.3	5195.7	4874.6	4134.8	3771.2
ZTW	2736.3	4962.9	4689.7	3908.1	3538.4	2288.5	4433.9	4079.3	3436.6	3124.0
SEm±	2.70	3.06	4.38	5.25	2.91	3.08	3.90	5.61	5.66	3.74
CD (P=0.05)	10.59	12.00	17.18	20.60	11.44	12.09	15.30	22.01	22.24	14.69
Nutrient management treatments										
Control ($N_0P_0Zn_0$)	2070.4	2458.7	2859.7	2196.9	1913.8	1597.0	1892.2	2196.2	1683.1	1458.5
RDN ($N_{120}P_{25.8}$)	1984.8	2349.3	2679.3	1976.2	1713.8	1343.3	1581.7	1778.0	1270.7	1101.0
RDN + Zn dose	3833.8	8417.5	7417.5	6491.5	5998.0	4485.7	9006.4	7968.2	7003.0	6462.7
75% RDN ($N_{90}P_{19.35}$)	2024.8	2398.2	2758.5	2065.3	1792.7	1427.9	1684.0	1917.7	1414.3	1211.9
75% RDN + Zn	3888.7	8468.2	7498.4	6604.3	6111.4	4579.9	9092.2	8077.2	7144.3	6586.7
75% RDN + MI1	1992.8	2359.1	2694.4	1992.0	1731.4	1363.5	1606.1	1810.3	1299.9	1127.3
75% RDN + MI1+ Zn	3840.5	8423.8	7426.8	6505.3	6011.9	4495.2	9015.2	7979.6	7018.6	6474.9
75% RDN + MI2	1990.2	2355.9	2689.0	1986.8	1726.1	1355.9	1597.0	1798.6	1288.1	1116.4
75% RDN + MI2 + Zn	3834.5	8418.3	7420.4	6496.4	6003.1	4489.3	9009.8	7972.7	7009.9	6467.8
SEm±	3.35	3.88	5.85	7.14	4.38	5.25	6.45	8.98	9.01	6.86
CD (P=0.05)	9.53	11.05	16.64	20.31	12.46	14.93	18.34	25.52	25.62	19.52

MI1: *Anabaena* sp. (CR1) + *Providencia* sp. (PR3); MI2: *Anabaena-Pseudomonas* biofilmed formulation; Zn*: Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; RDN: Recommmended dose of nutrients

activity in soil is contributed by externally applied microbial inoculation. Dehydrogenase activity also showed positive correlation with Zn concentration at 60 and 90 DAS (Fig 2).

DTPA-extractable Zn in soil

Soil Zn level was increased at 30 DAS as compared to initial level in both Zn applied and Zn omitted treatments in first year (Table 2). Increase was more in Zn applied treatment (4580-4590 g/ha) as compared to Zn omitted treatment (366-388 g/ha). Soil Zn increased from 30 to 60 DAS in Zn omitted treatments by 300-400 g/ha while it decreased by about 996-1,000 g/ha in Zn applied treatment which might be due to both uptake and fixation of Zn in Zn applied treatments. Among CEMs, CDW and SWI were statistically superior to ZTW at 30 DAS; while at 60 DAS all methods remained on par to each other. Among nutrient management treatments, 75% recommended dose ($N_{90}P_{19.35}$) + Zn (8468 and 7498 g/ha) recorded significantly higher soil Zn at 30 and 60 DAS among Zn applied treatments which might be due to lower Zn uptake than other treatments even though application rate was remained similar. This higher soil Zn content showed the positive effect of Zn fertilization on soil DTPA-extractable Zn content of soil. Increase in soil Zn due to Zn fertilization was also reported by Abid *et al.* (2013).

Among Zn omitted treatments, control was statistically superior both at 30 and 60 DAS to 75% RDN. These both the treatments had significantly higher soil Zn than other Zn omitted treatment. The lowest soil available Zn was recorded in 100% RDN which might be due to higher uptake than rest of the treatments and it also showed the relationship between soils DTPA-extractable Zn with Zn uptake in plant. Soil available Zn at 90 DAS was lower than at 60 DAS in both Zn applied and Zn omitted treatments. Decrease was more in 100% RDN + Zn (926 g/ha) and least in control (662 g/ha). This decrease was continued up to the harvest of wheat in all treatments. Compared to soil available Zn at start of wheat crop and at harvest, there was increase in available soil Zn by 856.7, 855 and 802 g/ha in CDW, SWI and ZTW, respectively. Increase in available soil Zn was higher in both CDW and SWI than ZTW which was due to higher uptake in ZTW. Trend in CEMs and nutrient management treatments at 90 DAS and at harvest was similar to that of 60 DAS with highest soil available Zn in CDW and the lowest in ZTW; while among nutrient management treatments, the highest available soil Zn in 75% RDN + Zn (6604 and 6111.4 g/ha) and lowest in 100% RDN (1,976 and 1,713 g/ha) at both 90 DAS and at harvest. Soil Zn content in 75% RDN + MI1 or MI2 was significantly lower than 75% RDN which was because of higher uptake in MI1 and MI2 applied treatment with 75% RDN than application of only 75% RDN. This dictates the role of microbial inoculation in deciding the soil Zn status.

Soil available Zn at the start of second wheat crop was higher than that of first season wheat crop by 170 g/ha in both CDW and SWI; while it was lower by 448 g/ha in

ZTW. Soil Zn was higher at 30 DAS as compared to initial available Zn level which was mainly due to application of Zn. Available soil Zn at 60 DAS however, decreased by 319, 321 and 354 g/ha in CDW, SWI and ZTW, respectively as compared to 30 DAS. Among CEMs, CDW and SWI remained on par to each other at 30 and 60 DAS and recorded significantly higher soil Zn than ZTW. Among Zn applied treatments, 75% RDN + Zn (9092 and 8077 g/ha) recorded significantly higher soil Zn than all other Zn applied treatments and among Zn omitted treatments, control (1892 and 2196 g/ha) recorded significantly higher soil Zn at 30 and 60 DAS being statistically superior to 75% RDN. The lowest soil Zn was observed in RDN which remained inferior to all other treatments. Soil available Zn at 90 DAS was lower than 60 DAS by 739 g/ha in both CDW and SWI and 642 g/ha in ZTW; while soil Zn at 90 DAS was higher than soil Zn at harvest of wheat by 363 g/ha in both CDW and SWI and 312 g/ha in ZTW. The trend among CEMs and nutrient management treatments at 90 DAS and at harvest was similar as that of 60 DAS with the highest and the lowest available soil Zn was recorded with 75% RDN + Zn (7144 and 6586 g/ha) and 100% RDN (1270 and 1011 g/ha), respectively at both 90 DAS and at harvest.

Balance of soil DTPA-extractable Zn

DTPA-extractable Zn in soil at initial stage was higher in second year by 1142; 1140 and 385 g/ha in CDW, SWI and ZTW, respectively which showed the positive effect of Zn fertilization on soil Zn status and also reflect the relationship between plant total Zn uptake and DTPA-extractable available Zn in soil as ZTW had lowest Zn content and same method has higher total Zn uptake in plant (Table 3). Soil Zn content at initial stage was similar among CEMs in first year; while it was significantly higher in both CDW and SWI than ZTW in second year. Calculated balance and actual Zn present in soil in CEMs also had similar trend as that of initial available soil Zn content during second year. Calculated balance was found higher than actual DTPA-

Table 3 Effect of crop establishment methods and nutrient management options on DTPA-extractable zinc (Zn) balance in soil

Treatment	Initial DTPA-extractable Zn in soil (g/ha)		Zn applied through fertilizer (g/ha)		Total Zn present in soil (g/ha)		Total Zn uptake (g/ha)		Calculated balance (g/ha)		Actual Zn present in soil after harvest (g/ha)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
Crop establishment methods												
CDW	1904	3046.4	2222	2222	4126	5268.6	266.7	245.7	3859.5	2800.7	3732.9	3773.9
SWI	1904	3044.3	2222	2222	4126	5266.5	268.5	246.5	3857.8	2797.8	3729.4	3771.2
ZTW	1904	2288.5	2222	2222	4126	4510.8	334.7	309.6	3791.5	1978.9	3538.4	3124.0
SEm±	–	3.08	–	–	0	3.1	1.26	1.23	1.26	4.3	2.91	3.74
CD (P=0.05)	–	12.09	–	–	0	12.1	4.93	4.82	4.93	16.8	11.44	14.69
Nutrient management treatments												
Control ($N_0P_0Zn_0$)	1904	1597.0	0	0	1904	1597.0	161.0	144.7	1743.0	1452.3	1913.8	1458.5
RDN ($N_{120}P_{25.8}$)	1904	1343.3	0	0	1904	1343.3	305.5	281.1	1598.5	1062.2	1713.8	1101.0
RDN + Zn* dose	1904	4485.7	5000	5000	6904	9485.7	349.6	316.8	6554.4	4168.9	5998.0	6462.7
75% RDN ($N_{90}P_{19.35}$)	1904	1427.9	0	0	1904	1427.9	255.3	242.7	1648.7	1185.2	1792.7	1211.9
75% RDN + Zn	1904	4579.9	5000	5000	6904	9579.9	263.4	246.7	6640.6	4333.2	6111.4	6586.7
75% RDN + MI1	1904	1363.5	0	0	1904	1363.5	294.1	273.0	1609.9	1090.5	1731.4	1127.3
75% RDN + MI1+ Zn	1904	4495.2	5000	5000	6904	9495.2	339.1	309.8	6564.9	4185.4	6011.9	6474.9
75% RDN + MI2	1904	1355.9	0	0	1904	1355.9	297.4	277.2	1606.6	1078.7	1726.1	1116.4
75% RDN + MI2 + Zn	1904	4489.3	5000	5000	6904	9489.3	344.4	313.5	6559.6	4175.8	6003.1	6467.8
SEm±	–	5.25	–	–	0	5.3	2.85	2.58	2.85	7.8	4.38	6.86
CD (P=0.05)	–	14.93	–	–	0	14.9	8.11	7.35	8.11	22.1	12.46	19.52

MI1: *Anabaena* sp. (CR1) + *Providencia* sp. (PR3); MI2: *Anabaena-Pseudomonas* biofilmed formulation; Zn*: Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; RDN: Recommended dose of nutrients

extractable Zn present in soil at harvest during first year by 127 to 253 g/ha which could be attributed its fixation in soil. During second year calculated balance was found lower by 973 to 1145 g/ha than actual DTPA-extractable Zn. This was due to higher total application rate than sum of total uptake and amount of applied Zn get fixed in other form which was not extractable by DTPA.

DTPA-extractable Zn in soil at initial stage and at harvest was found significantly higher in all Zn applied treatment than Zn omitted ones which signifies the role of Zn fertilization on increasing soil DTPA-extractable Zn in soil. Among Zn applied treatments, 75% RDN + Zn had significantly higher DTPA-extractable Zn at initial stage, in calculated balance and actual Zn present in soil at harvest which was due to lower uptake of Zn than rest of treatments even though application rate was similar in all treatments. This also indicates the role of application of recommended dose of N and P on soil DTPA-extractable Zn status. Among Zn omitted treatments, control had the highest and 100% RDN recorded the lowest soil DTPA-extractable Zn at initial stage and at harvest (both in calculated balance and actual Zn present in soil at harvest) which was also due to variation in Zn uptake as influenced by various rate of N and P application, even though Zn was not applied in all treatments. This also indicates the role of application of major nutrients (N and P) in DTPA-extractable Zn status of soil.

Results of present investigation indicate the positive and significant role of CEMs, N and P application and Zn fertilization on Zn nutrition of wheat. Zero tillage wheat was found superior than CDW and SWI in Zn nutrition of wheat and at the same time this method also had lower level of soil DTPA-extractable Zn in soil due to higher uptake. Increase in Zn concentration and uptake along with increase in soil DTPA-extractable Zn at harvest in both years of study over initial soil Zn content showed the positive role of Zn fertilization in plant and available soil Zn content. Application of Zn with 75% RDN + MI1 or MI2 remained on par with application of Zn with 100% RDN in enhancing concentration and uptake of Zn in soil which showed the positive role of microbial inoculation in Zn nutrition of wheat.

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