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EFFECT OF ZINC APPLICATION ON ZINC INFLUX, UPTAKE AND ITS DEPLETION IN THE RHIZOSPHERE OF RICE, MAIZE AND MOONG CROPS UNDER ZINC DEFICIENT SOIL

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ABSTRACT

Crops exhibit considerable variation in zinc acquisition efficiency, particularly in zinc-deficient soils this diversity creates a prime opportunity to identify and develop varieties that excel under Low-Zn conditions. Concerning the widespread deficiency of zinc in Punjab since few decades a pot experiment was conducted in zinc deficient sandy loam soil to study the “Effect of zinc application on zinc influx, uptake and its depletion in the rhizosphere of rice and maize under zinc deficient soil”. The treatments were Zn₀ (control), Zn₅ (5 mg Zn kg⁻¹ soil), Zn₁₀ (10 mg Zn kg⁻¹ soil) and Zn₅₀ (50 mg Zn kg⁻¹ soil) applied using ZnSO₄. Under zinc deficiency conditions rice, maize and moong produces 86%, 76% and 71 % and 64 %, 58 % and 61 % of their maximum SDW having zinc content 27.56, 33.56, and 18.20 mg Zn kg⁻¹ shoot and 15.23, 22.60 15.16 mg Zn kg⁻¹ shoot after 30 and 60 DAG respectively. If relative shoot yield considered as a measure of efficiency maize was more zinc efficient than rice and moong. Maize was more zinc efficient because it can maintain 4.7 and 6 times more zinc than rice and moong respectively. With the application of Zn the root weight, shoot weight root length, root surface area, zinc influx, shoot demand were significantly affected. No significant change was observed in terms of soil pH, electrical conductivity and organic carbon before and after the experiment. Among zinc treatments, 10 mg Zn kg⁻¹ soil application gives highest results which was reported at par with 5 mg Zn kg⁻¹ soil.

Keywords : Zinc, influx, depletion, rhizosphere, rice, maize, days after germination.

Introduction

The rhizosphere was defined as the zone of soil that is under the influence of plant roots. But in current model, rhizosphere is the millimetric zone surrounding the roots where we find rhizodeposits, which include sloughed-off root cells, mucilage, and root exudates such as organic acids, amino acids, and secondary metabolites (Porte *et al.*, 2020). The production of food and fibre, as well as the acquisition of resources associated with it, are significantly impacted by the rhizosphere's operation (York *et al.*, 2016), additionally with reference to carbon sequestration and climate change (Keiluweit *et al.*, 2015). The physical (pore volume, connectedness and aggregation) and biogenic (surface coatings, mineral associated carbon) soil structure is shaped and organized by roots and micro-organisms in the rhizosphere, which at the soil

profile size regulates infiltration, water storage and aeration. The plant species and soil type have an impact on the rhizosphere microbiome, which is essential for plant health (Philippot *et al.*, 2013; Berg *et al.*, 2017; Vetterlein *et al.*, 2020). In the rhizosphere micronutrient availability is controlled by soil and plant properties, and interactions of roots with microorganisms and the surrounding soil. Rhizosphere soil acidification increases the mobilization of micronutrients e.g. For Zn, 100-fold increase in solubility for each unit of pH decreases (Rengel, 2015). The concentration of zinc available to plants is low in soils with different characteristics: high and low pH, high and low organic matter, calcareous, sodic, sandy, wetland or ill-drained, limed acid soils etc. Zinc deficiency is common in rice on neutral to alkaline pH soils containing more than 10 g organic matter kg⁻¹ and in calcareous soils used for upland rice production

(Rehman *et al.*, 2012). Plant species, as well as the genetics of a specific species, have an impact on the pH of the rhizosphere (Tanikawa *et al.*, 2014).

The different mechanisms active in the rhizosphere and within the plant system have been linked to zinc efficiency in conditions of deficiency. Long and delicate plant roots with an architecture that favours the extraction of zinc from larger soil volumes, as well as increased root production of the phytosiderophore zinc mobilizing in the rhizosphere, encourage the uptake of zinc as the zinc phytosiderophore complex (Dotaniya and Meena, 2015).

The diversity of micro-organisms is greater near the rhizosphere. It decreases as the distance from the rhizoplane increases (Dotaniya and Meena, 2015). The interaction of plant nutrients in the soil and plant exudates changes the microclimate of the rhizoplane (Shukla *et al.*, 2013). Certainly, arbuscular mycorrhizal fungi can increase the accumulation of several nutrients, including zinc, but their actual importance varies with factors such as fertilization practices, soil and crop characteristics, and management (Srinivasagam *et al.*, 2013).

Zinc is one of the essential micronutrient. Zinc deficiency is widely reported nowadays. About half of the world's soils are naturally low in zinc (Hacisalihoglu, 2020). In soil, its values fluctuate depending upon the parent material, most agricultural soils contain zinc ranging from 10-300 mg kg⁻¹ soil (Noulas *et al.*, 2018). In plants, it is required for production of superoxide dismutase and it is constituent of carbonic anhydrase, alcoholic anhydrase, ribonucleic acid polymerase (Xi-Wen *et al.*, 2011, Rehman *et al.*, 2012). It serves a role in carbohydrate and phosphorus metabolism (Yadavi *et al.*, 2014). About 49% of world's population does not meet its physiological zinc requirements which are 10.4 to 15.3 mg Zn day⁻¹ and 14.4 to 23.3 mg Zn day⁻¹ for adult women and men, respectively (Ahsin *et al.*, 2020). The major crops showing zinc deficiency are beans, citrus, flax, fruit trees, grapes maize, onions, rice, sorghum, sweetcorn (Alloway, 2008).

In Punjab, as soils of Punjab are alkaline with high CaCO₃ content which is predisposing factor of zinc deficiency. Thus, very little fraction of available zinc is present in soil for plant uptake. About 4.20, 19 and 12.1 % soils of Punjab are acute deficient, deficient and latent deficient respectively of Punjab soils are affected by Zinc deficiency (Shukla *et al.*, 2021). Rice-wheat, maize-wheat and cotton-wheat are the three predominant cropping systems, being

practiced by a majority of farmers in different agro-climatic zones. Due to intensive cultivation the production of food grains has increased resulting in depletion in huge amount of macro as well as micro nutrients. In Punjab, micronutrient deficiencies started emerging at an alarming rate with the adoption and spread of intensive agriculture, imbalanced use of macronutrient fertilizers and decrease in use of organic manures and reduced recycling of crop residues (Dhaliwal *et al.*, 2011).

The objective of this study was to understand the Zn uptake mechanism of rice, maize and moong crops and its depletion in the rhizosphere. Different soil and plant parameters were calculated for both the crops to check the Zn influx by the crops.

Material and Methods

A pot culture experiment was conducted to determine zinc uptake by rice, maize and moong. The soil taken for the experiment is zinc deficient soil having pH of the soil was 8.13, low in organic carbon (0.36%) with 150, 15 and 260 kg ha⁻¹ of available N, P and K respectively. The micronutrient cations *viz.*, Zn, Cu, Fe and Mn were 0.50, 0.60, 12.36 and 7.45 mg kg⁻¹. Pots contains 5 kg soil and is treated with 0, 5, 10, and 50 mg Zn kg⁻¹ soil. Each pot was given with basal dose of NPK as per the crops given in package and practices of Punjab Agricultural University, Ludhiana. The crops are harvested at two growth stages. First at 30 (DAG) days after germination and second at 60 (DAG). The treatments are replicated 6 times. Three were used for first growth stage and remaining three were used for second growth stage. 10 and 4 plants were maintained for first and second growth stage respectively. Moisture of soil was maintained at 20%. The pots were measured daily to check the water loss and to maintain the soil moisture level. After the harvesting the shoots of the crops was washed properly with distilled water and fresh shoot weight was recorded. Then shoots were dried in hot air oven at 60 ± 5°C until a constant weight is recorded. Then the shoot dry weight was recorded. Dried samples were ground in a stainless steel mixer and digested in di-acid mixture (HNO₃: HClO₄ – 4:1) and were analyzed at (MPAES) Micro Plasma Atomic Emission Spectrometer.

Roots of the plants were separated carefully from soil by washing with distilled water over the sieve. After washing carefully, the roots were dried by keeping between filter paper to remove excess water from the surface of roots and fresh root weight was recorded.

DTPA extractable zinc was determined by using the method given by Lindsay & Norvell (1967), pH and EC by (Jackson, 1967) and OC by using the method of Walkley & Black (1934).

Zn influx (I_n) was calculated from formula of Williams (1948):

$$I_n = \frac{U_2 - U_1}{RL_2 - RL_1} \frac{\ln(RL_2 / RL_1)}{t_2 - t_1}$$

I_n is Zn influx ($\text{mol cm}^{-1} \text{s}^{-1}$), U is Zn content (mol plant^{-1}), RL is root length (cm), t is time (seconds), Subscript 1 and 2 refer to previous and current harvest.

Root length (RL) was measured by the line interception method of Tennant (1975), using the formula:

$$RL = 11/14 \times N \times G$$

RL is root length (cm), N is total number of intercepts of roots with vertical and horizontal grid lines, G is grid square dimensions (cm)

Root radius was calculated by the formula:

$$r_o = (\text{Fresh root weight} / \pi \cdot \text{root length})^{1/2}$$

Root surface area was calculated as follows:

$$RA = 2 \pi r_o RL$$

RA is root surface area (cm^2), RL is root length (cm), r_o is root radius (cm)

Relative root growth rate:

$$k = \frac{\ln(RL_2 / RL_1)}{t_2 - t_1}$$

k is relative root growth rate (s^{-1}), RL_1 and RL_2 (cm) are root length at time t_1 and t_2 (s)

Shoot demand: Amount of shoot produced per second per unit of root

$$SD = \frac{W_2 - W_1}{RL_2 - RL_1} \frac{\ln(RL_2 / RL_1)}{t_2 - t_1}$$

SD is shoot demand ($\text{mg cm}^{-1} \text{s}^{-1}$), W is dry matter yield (mg), RL is root length (cm), t is time (seconds), Subscript 1 and 2 refer to previous and current harvest.

Zn uptake was calculated using the formula:

$$\text{Zn uptake (g pot}^{-1}\text{)} = \frac{\text{Nutrient content (mg kg}^{-1}\text{)} \times \text{Biomass (g pot}^{-1}\text{)} \square}{1000}$$

Results and Discussion

Under no-Zn treatment rice and maize shows Zinc deficiency symptoms after 21 and 28 days after germination and mild Zinc deficiency comes in moong after 40 days of germination. Plant growth and Zn uptake differed in all the crops at both growth stages (Table 1, Fig. 1). There was a significant increase in shoot dry weight (SDW), root dry weight (RDW), root surface area and Zn uptake by the crops. Zn level @ 5 mg Zn kg^{-1} soil gives maximum shoot dry weight in rice which was statistically at par with 10 mg Zn kg^{-1} soil after 60 days of growth. Zn application at the rate 50 mg Zn kg^{-1} soil gives maximum shoot dry weight which was also at par with 5 and 10 mg Zn kg^{-1} soil in both maize and moong. There was 1.82, 1.70 and 1.31 times increase in dry shoot weight of rice, maize and moong respectively at second growth stage with the application of zinc. The improvement due to Zn application in crops indicates more requirement of Zn by these crops. The increase in fresh shoot weight of rice was also found by (Farooq *et al.*, 2022) for Mg

The application of 5 mg Zn kg^{-1} soil gives significantly highest result for root dry weight (RDW) in rice which was at par with 10 and 50 mg Zn kg^{-1} soil and for maize at 10 mg Zn kg^{-1} soil, 5 and 50 mg Zn kg^{-1} soil levels are at par with it (Table 1). There was 1.71 and 1.57 times increase in dry shoot weight of rice and maize respectively. This may be due to because maize has bigger root system as compared to rice and moong. The dry weight of root increased in all the crops due to Zn application at second growth stage. Similar results were also reported by Bashir *et al.* (2021) in maize.

The root surface area (RSA) of all crops was significantly increased by application of zinc as given in table 1. Maximum result was found at 5 mg Zn kg^{-1} soil application and minimum was found at control condition in rice. For maize maximum (RAS) was found at 10 mg Zn kg^{-1} soil application which was also at par with 5 and 50 mg Zn kg^{-1} soil. For moong maximum root surface was found at 50 mg Zn kg^{-1} soil which was at par with 5 and 10 mg Zn kg^{-1} soil and minimum was found at control in both the stages. Higher surface area was found in maize. This might be because maize has extensive root system. With the application of Zn, an increase in root surface area was noticed which could be due to increment in root length. Similar results were found by Verma *et al.* (2021).

Root length of all the crops varied widely under low and high Zn supply (Fig. 1). Under zinc deficient conditions root length of maize was 2 fold higher than rice and 7 fold higher than moong. Zinc application has

significantly increased the root length in all the crops. Root length increased because of development of lateral roots. Similar results were found by (Akman and Topal, 2014)

Due to increase in root length relative root growth rate and shoot demand of both the crops by applying zinc in soil is increased (Table 3). Zinc acquisition load created by shoot growth on each cm of root is called as shoot demand. Under zinc deficient condition relative root growth rate and shoot demand was 2 and 2.03 times greater in maize as compared to rice and 6 and 3 times greater in maize as compared to moong respectively. These factors increased due to increases in root length.

Zinc influx was significantly increased by the application of zinc as compared to low Zn conditions (Fig 2). Maize has 4.7 times and 6 times more zinc influx than rice and moong respectively. Because of maize's extensive root system. There was no

significant change in pH, EC and OC of all the crops (Table 4).

DTPA extractable zinc was measured after both the harvests of the crops (Fig. 2). DTPA extractable zinc of soil was increased in which zinc was applied. The same results were found by Kumar *et al.* (2014). The 50 mg kg⁻¹ soil zinc treatment was taken to check the toxicity effect of zinc on crops and the zinc retaining capacity of soil. No toxic effect was found on both the crops. Zinc content above 150 mg kg⁻¹ soil leads to reduction in plant growth (Noulas *et al.*, 2018).

Conclusion

Maize has 4.7 and 6 times more Zn influx than rice and moong under zinc deficient conditions. Because maize has extensive root system than rice. The influx of the crops was increased with the application of zinc. The zinc range of 5 mg kg⁻¹ soil was sufficient to improve plant parameters and zinc content of both the crops. Under zinc deficient conditions crops with more profound root systems performs well.

Table 1: Shoot dry weight (SDW), root dry weight, root surface area (RSA) and Zn uptake of rice, maize and moong grown on Zn deficient soil with and without zinc application

Crop	Zn applied (mg kg ⁻¹ soil)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Root surface area (cm ² plant ⁻¹)	Zn uptake (10 ⁻¹ g plant ⁻¹)
First Harvest After 30 Days of Growth					
Rice	0	0.19	0.08	0.13	0.27
	5	0.23	0.15	0.20	0.53
	10	0.22	0.14	0.19	0.60
	50	0.22	0.15	0.19	0.78
Maize	0	1.00	0.67	1.31	1.82
	5	1.30	1.27	1.91	9.51
	10	1.31	1.29	1.95	9.69
	50	1.30	1.28	1.94	10.27
Moong	0	0.23	0.06	0.09	0.23
	5	0.32	0.07	0.10	0.47
	10	0.31	0.09	0.13	0.59
	50	0.31	0.11	0.13	0.76
LS D(0.05) Between crops Zn levels Crops×Zn levels		NS 0.0026 0.1330	0.1333 0.1539 0.2665	0.1376 0.1589 0.2753	0.7373 0.8520 1.4757
Second Harvest After 60 Days of Growth					
Rice	0	1.44	1.03	2.10	1.46
	5	2.63	1.76	2.81	4.28
	10	2.32	0.66	2.73	4.42
	50	2.24	0.17	2.84	5.48
Maize	0	2.77	5.17	9.69	6.73
	5	4.70	8.15	13.11	41.80
	10	4.75	8.53	13.78	47.37
	50	4.80	8.37	13.42	56.57
Moong	0	1.93	0.14	0.27	1.19
	5	2.53	0.19	0.31	2.64
	10	3.20	0.28	0.31	4.51
	50	3.41	0.29	0.42	5.53
LS D(0.05) Between crops Zn levels Crops×Zn levels		0.2805 0.3239 0.5611	0.2799 0.3232 0.5598	0.3871 0.4470 0.7742	1.4447 1.6682 2.8894

Table 2: Root length, root radius, and average distance between neighboring roots of rice, maize and moong grown on Zn deficient soil with or without zinc application

Crop	Zn applied (mg kg ⁻¹ soil)	Root length (cm)	root radius (10 ⁻³ cm)	Average distance between neighboring roots (cm)	Shoot Zn content	
First Harvest After 30 Days of Growth						
Rice	0	91.43	0.23	3.90	27.56	
	5	176.80	0.18	2.80	36.86	
	10	180.70	0.17	2.77	44.73	
	50	193.60	0.16	2.68	60.36	
Maize	0	253.80	0.82	3.48	33.56	
	5	337.50	0.90	2.02	103.60	
	10	337.70	0.92	2.11	125.20	
	50	339.60	0.91	2.02	141.70	
Moong	0	30.83	0.46	6.73	18.20	
	5	54.62	0.29	5.05	35.06	
	10	56.75	0.36	4.95	43.96	
	50	55.92	0.37	4.99	58.93	
LSD (0.05) Between crops Zn levels Crops×Zn levels		14.606 16.865 29.212	0.0656 0.0757 0.1312	0.00561 0.00648 0.0112	4.6228 5.3380 9.2456	
	First Harvest After 30 Days of Growth					
	Rice	0	300.80	1.11	2.16	15.23
5		630.80	0.71	1.48	26.64	
10		638.50	0.68	1.47	30.22	
50		645.50	0.70	1.46	36.66	
Maize	0	600.30	2.57	1.52	22.60	
	5	1250.00	1.67	1.05	85.63	
	10	1247.00	1.76	1.05	97.73	
	50	1327.00	1.61	1.02	112.70	
Moong	0	93.86	0.46	3.85	15.16	
	5	153.70	0.32	3.00	25.93	
	10	164.40	0.30	2.91	34.40	
	50	164.90	0.40	2.90	31.56	
LSD (0.05) Between crops Zn levels Crops×Zn levels		54.944 63.444 109.89	0.1221 0.1409 0.2441	NS NS NS	2.4829 2.8670 4.9658	

Table 3 : Relative root growth rate and Shoot demand of rice, maize and moong grown on Zn deficient soil with or without zinc application

Crop	Zn applied (mg kg ⁻¹ soil)	Relative root growth rate (10 ⁻⁴ s ⁻¹)	Shoot demand (10 ⁻⁴ mg cm ⁻¹ s ⁻¹)
First Harvest After 60 Days of Growth			
Rice	0	1.57	7.17
	5	3.30	13.80
	10	3.34	12.20
	50	3.38	11.70
Maize	0	3.14	14.50
	5	6.54	24.60
	10	6.53	24.90
	50	6.95	25.20

Moong	0	0.49	4.66
	5	0.81	6.11
	10	0.86	7.56
	50	0.86	7.88
LSD (0.05)			
Between crops		0.35	1.25
Zn levels		0.41	1.44
Crops×Zn levels		0.71	2.50

Table 4: Effect of Zn application on pH, EC and OC of soils of different crops

Crop	Zn applied (mg kg ⁻¹ soil)	pH	EC	OC
First Harvest After 30 Days of Growth				
Rice	0	8.26	0.30	0.36
	5	8.27	0.32	0.34
	10	8.27	0.34	0.36
	50	8.24	0.34	0.34
Maize	0	8.31	0.31	0.35
	5	8.29	0.33	0.35
	10	8.26	0.37	0.36
	50	8.34	0.34	0.35
Moong	0	8.23	0.29	0.36
	5	8.21	0.31	0.35
	10	8.21	0.32	0.35
	50	8.20	0.32	0.34
LSD (0.05)				
Between crops		NS	NS	NS
Zn levels		NS	NS	NS
Crops×Zn levels		NS	NS	NS
First Harvest After 30 Days of Growth				
Rice	0	8.20	0.33	0.37
	5	8.22	0.35	0.36
	10	8.21	0.30	0.35
	50	8.27	0.28	0.35
Maize	0	8.29	0.32	0.35
	5	8.25	0.31	0.35
	10	8.29	0.34	0.36
	50	8.36	0.32	0.36
Moong	0	8.22	0.28	0.35
	5	8.19	0.29	0.36
	10	8.19	0.31	0.36
	50	8.18	0.31	0.36
LSD (0.05)				
Between crops		NS	NS	NS
Zn levels		NS	NS	NS
Crops×Zn levels		NS	NS	NS

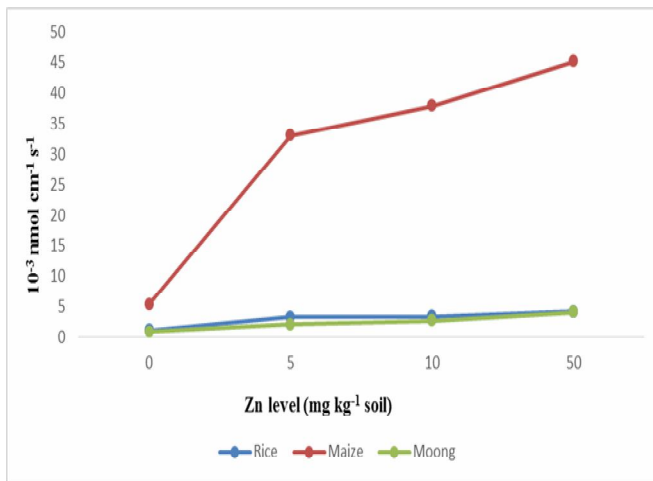


Fig. 1: Effect of Zn application on Zinc influx of rice, maize and moong

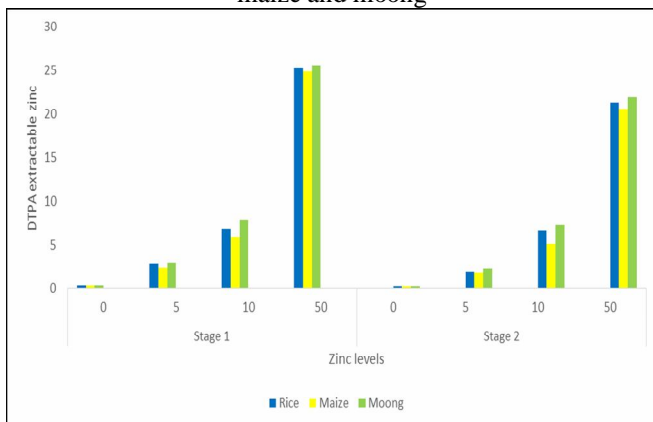


Fig. 2: Effect of zinc application on DTPA extractable zinc

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