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Authors' contributions

This work was carried out in collaboration among all authors. Author SJ designed the study, managed the analyses of the study performed the statistical analysis, wrote the protocol, authors GST and BLS managed the literature and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Field experiment was conducted at the research Farm of JNKVV, Jabalpur (M.P) during the Kharif season of 2018 with three main treatments as single year application, alternate and each year zinc addition and five levels of Zn (0, 2.5, 5, 7.5 and 10 kg ha-¹ as sub treatments in a split plot design. Results revealed that alternate year Zn application increased seed yield by 1.48 t ha-¹ over single year. The difference recorded in between alternate year and single year Zn application was 0.33 t ha-¹. Treatment 5.0 kg Zn ha-¹ had significantly increased seed yield by 1.43 t ha-¹ over control. The difference recorded in between 5.0 kg Zn ha-¹ and in control Zn application was 0.38 t ha-¹. The each year Zn application and 10 kg Zn ha-1 gave the highest protein (40.30%) and oil content (20.17%) in seed. Results explained that the higher release of Zn pools were observed with the each year Zn application and 10 kg Zn ha-¹. The order of prevalence of Water soluble and exchangeable-Zn< Complexed-Zn<Amorphous Sesquioxide < Crystalline Sesquioxide bound-Zn< Residual-Zn.

Keywords: Zinc fertilization; zn uptake; yield; quality; soybean; zinc pools.



1. INTRODUCTION

Soybean (Glycine max (L.) Merril) is serves as an oilseed and pulse crop and nutritionally, it is the richest and cheapest source of protein (40-42%) and amino acids, vitamins, minerals (5%), fats (15-20%), carbohydrate (30%) and dietary fiber. Daidzein and genistein, which are isoflavone aglycones are mainly found in soybeans and soy products [1]. Zinc is an activator of enzymes in plant viz, carbonic anhydrase, alcohol dehydrogenase, glutamic dehydrogenase and chlorophyll formation. A widespread form of anti nutrients, constituents like Phytic Acid (PA). Phytic acids chelate several mineral elements, especially Zn, Fe, Ca, Mg and Mo and interfere with their absorption and utilization. Legumes have low input requirement and enhance diversification in crop rotation [2] (Fazekašová et al., 2011). In India, it is grown on an area of 10.57 M ha with a production 9.14 Mt and productivity of 901 kg ha-¹. However it is grown on an area of 5.91 M ha with a production of 4.91 Mt and productivity of 905 kg ha⁻¹ in Madhya Pradesh [3].

Zn plays an essential role in plant metabolism such as gene regulation and expression, protein synthesis, carbohydrate metabolism, phytohormone action, seed photosynthesis, production and defense against plant disease [4,5]. Zn acts on activity of various enzymes including RNA polymerase, carbonic anhydrase, alcohol dehydrogenase, glutamate dehydrogenase and superoxide dismutase (Cu/Zn-SOD) [6]. The decreased photosynthetic activity of Zn-deficient plants may be due to inhibition of carbonic anhydrase activity, decreased chlorophyll content and changes in chloroplast structure. Consequently, soils with low Zn availability exhibit lower yield potential and negatively affect the nutritional quality of the harvested grain [7]. Zinc is estimated that about 10% of all the proteins in the human body, corresponding to nearly 3000 proteins, are Zndependent [8,9]. However, it is estimated that about 50% of the soils used for grain production worldwide are deficient in plant-available Zn [6]. Zn deficiency in human might be due to longterm consumption of staple diets from crops grown in Zn-deficient soils [10,11].

The total Zn in normal soils ranged from 10 to 300 mg kg⁻¹ with a mean value of 50 mg kg⁻¹ [11]. Part of the soil total Zn (30 to 60%) may be as plant-unavailable forms, trapped in organic matter or adsorbed on mineral colloids [12]. The

soil factors which control the amount of Zn sorption-desorption from/into the soil solution, the total Zn content, soil texture, soil organic C content calcium carbonate content, redox conditions, soil solution soil clay mineralogy pH soil temperature microbial activity in the rhizosphere, soil moisture, root system anatomy, fertilizer types used, accompanying ion of the source of Zn used and contaminants that may be found in the fertilizers [13,14].

The biofortification of the Zn content via soil fertilization involves many physiological steps A better understanding of these steps should increase our ability to enhance the Zn content of grain via genetic and agronomic biofortification. The seed removal is the main factor responsible for the nutrient export in crops. Nonetheless, little is known about the nutrient exports in soybean with genetic potential for high seed yield. Even when new cultivars with a strong genetic capacity for Zn absorption are developed, such absorption greatly depends on the size of the available Zn pool in soil. Fertilization of soil with Zn, which is usually applied as ZnSO₄ 7H₂O, appears to be important for ensuring the success of [15]. Suitable biofortification and proper application methods of Zn fertilizer are still unclear. It has been reported that basal Zn fertilizer may have a strong residual effect, but in some soils, Zn can be fixed and is therefore not utilized by the crop.

Soybean is a major crop grown on Vertisols and associated soils in Madhya Pradesh. Due to inappropriate management practices such as use of straight NPK fertilizers, with free from Zn, not only limits crop productivity but also affect quality and human health. From review, still, there are few field studies so far study was conducted to study the effect of Zinc fertilization on yield, quality of soybean and zinc pools in a Typic Haplusterts.

2. MATERIALS AND METHODS

2.1 Experiment Location and Description of Materials

Field experiment was carry out at experimental farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India during 2018 in *Kharif* season (23°12' 57" N and 79°56' 49" E).The region has a semi-arid and sub-tropical climate, summer to cool winter with a mean annual temperature of 25.7°C and precipitation of 1115.10 mm. Soil details; which was analyzes in Jawaharlal Nehru Krishi Vishwa

Vidyalaya, Jabalpur micronutrient laboratory, medium black soil classified as Vertisol, with pH of 7.6 in soil 0.19 dS m⁻¹ electrical conductivity, 6.10 g kg⁻¹ organic carbon, 0.33 kg ha⁻¹ available Zn and bulk density of 56.9% clay, 17.8% silt, and 25.4% sand.

2.2 Treatments and Experimental Design

The experiment was studied in 2018 with three main treatments (periodicity of Zn application) as single year application (Zn applied during Kharif 2012) it was applied once, alternate year application (Zn applied during Kharif 2012, 2014, 2016 and 2018 it was applied alternate and each year application (Zn applied during Kharif 2012, 2013, 2014, 2015, 2016, 2017 and 2018 it was applied every year and five levels of Zn (0, 2.5, 5, 7.5 and 10 kg ha⁻¹ through ZnSO₄.7H₂O as sub treatments were applied to soybean. These fifteen treatments randomly allocated in split plot design with three replications. The basal dose of 20 kg N, 80 kg P₂O₅ and 20 kg K₂O ha⁻¹ was applied at the time of sowing because of legume crop so K requirement also very less.

2.3 Sampling and Analysis

Soil samples were collected from each treatment before sowing and after harvesting, five random cores were taken from a depth of 0 to 15 cm using a sampling auger. Subsamples were pooled to make composite samples. Composite samples were air-dried at room temperature, pulverized, sieved through a 2-mm sieve.

2.4 Physico-chemical Properties

The soil pH was measured in a soil: water ratio of 1: 2.5 using the pH meter and supernatant of same was used for electrical conductivity determination with the help of conductivity-meter Jackson [16]. Organic carbon in soil was determined using method as described by [17]. The calcium carbonate in soil was carried out using rapid back titration method as described by Jackson [16]. DTPA extractable micronutrients (i.e., zinc, copper, iron and manganese) were determined using Atomic Absorption Spectrophotometer (AAS) [18].

2.5 Plant Analyses

All soybean plants were harvested at crop maturity and grain yield was obtained. Seed protein, seed oil and seed carbohydrate analyzed by the AOAC 1995 method.

Next to this, seed protein, seed oil, seed carbohydrate and nutrient uptake was calculated by using the following formula:

Nutrient uptake (kg ha⁻¹) = Nutrient content (%) × yield (kg ha⁻¹)

Protein percent = N content in seed (%) x 6.25 (as a constant factor).

Protein yield (kg ha^{-1}) = Protein content (%) x Seed yield (q ha^{-1})

Oil yield (kg ha⁻¹) = Oil content (%) x Seed yield (q ha⁻¹)

2.6 Calculations and Statistical Analysis

All data were statistically analyzed with a twoway ANOVA procedure using the SPSS 19.0 software. The main effects and the interaction were analyzed using the F-value test.

3. RESULTS AND DISCUSSION

3.1 Yield Components and Yields of Soybean

The number of pods plant⁻¹ was significantly increased with increasing levels of Zn. The highest pod plant⁻¹ was recorded at 5.0 kg Zn ha⁻¹ and thereafter declines the pods plant⁻¹ 7.5 and 10 kg ha⁻¹. Moreover, the application @ 7.5 kg Zn ha⁻¹ produced significantly higher pods plant⁻¹ over control and 2.5 kg Zn ha⁻¹ application. Significant increase in seed test weight was observed over control but it was equal with higher levels. Minimum seed test weight was obtained with control (7.58 g) and highest was found at 5 kg Zn ha⁻¹ (8.08 g).

The data presented in (Table 1) illustrated that grain and stover yield for recommended at alternate year and 5kg Zn ha⁻¹. The lowest grain and stover yield was recorded in single year Zn application. The seed yield was observed (1.48 t ha-1) with alternate year addition of Zn, which increased to 1.15 and 1.29 t ha-1 with single and each year addition, respectively. However, single and each year application of Zn were found to be statistically at par. The interaction effect of Zn levels and its periodicity produced non-significant result. Application of 5 kg Zn ha⁻¹ recorded significant highest seed and stover yield which was on par with that obtained with 5, 7.5 and 10 kg Zn ha-1. The stover yield significantly increases with 5, 7.5 and 10 kg Zn ha-1. Moreover, the application 5 kg Zn ha⁻¹ produced significantly higher seed and stover yield over all

treatments with the increasing levels of Zn found statistically at par with stover yield highest at 5 kg Zn ha⁻¹.

The increase of yield attributes and yield might be due to beneficial effect of zinc on growth parameter and increased concentration of zinc in different plant part at various growth stages, involving in photosynthesis processes, respiration and nitrogen metabolism-protein synthesis. The findings are in agreement with Raghuwanshi et al., [19] also observed significant residual effect of zinc on succeeding in soybean-wheat cropping system. Nandanwar et al., [20] reported that grain and straw yield of soybean increased significantly with Zn 5.0 kg Zn application as compared to control. Pable et al.,[21] reported that zinc application increased the grain and straw yield of soybean over control. Similar results have been reported by Choudhary et al., [22], Nagajyothi et al. [23].

3.2 Zn uptake by Seed and Stover (g ha⁻¹)

Data revealed that the highest Zn uptake was recorded by seed and stover (78.21 and 35.11 g ha-1) with alternate year Zn application, which was significantly higher than the single year. Application with the alternate year Zn application was at par with each year Zn application. The lowest Zn uptake by seed and stover (58.00 and 26.26 g ha⁻¹, respectively) was recorded in single year application. The maximum and significant Zn uptake by stover (37.76 g ha⁻¹, respectively) was recorded with 10 kg Zn ha⁻¹ as compared to no Zn application and 2.5 kg Zn ha-1. However, 5, 7.5 and 10 kg Zn ha-1 Zn application increased Zn content. The minimum Zn uptake by seed and stover (52.21 and 23.77 g ha-1, respectively) were recorded in control.

The increases of Zn uptake with increasing levels of Zn might be due to increase of yield and Zn content in seed and stover yield as a result of increased Zn availability in the soil. Which caused higher metabolic and photosynthesis activity in plant resulted in greater uptake of Zn by crops and this leading to higher dry matter production, which led to higher Zn uptake by crops. The higher Zn uptake due to Zn application was also reported by Kanase et al. [24], Kobaree et al. (2011).

3.3 Quality of Seed of Soybean

3.3.1 Protein (%) in seed and protein yield

An investigation of the data presented in Table 3 showed that the protein content in seed was

observed to be 41.08% and 38.80% with alternate and each year application of Zn, respectively. Protein content in seed was significantly increased up to 10 kg Zn ha⁻¹ Zn levels 5.0, 7.5 and 10 kg Zn ha⁻¹ were showed equal protein content in seed. The highest protein content in seed of 40.30% was analyzed with application of 10 kg Zn ha⁻¹. The protein content in seed was (39.41, 39.92 and 40.06) 5 and 7.5 kg Zn ha⁻¹ which were similar protein content in seed. Similarly, protein yield of 575.46 and 530.94 kg ha⁻¹ was also observed with alternate and each year application of Zn, respectively. It was significantly increased up to 10 kg Zn ha⁻¹ but Zn levels 5.0, 7.5 and 10 kg Zn ha-1 were showed equal protein yield. The highest protein content of 40.30% was analyzed with application of 10 kg Zn ha⁻¹. The protein yield in seed was (488.03, 571.55 and 562.31 kg ha⁻¹) 2.5, 5.0 and 7.5 kg Zn ha⁻¹ which were similar protein yield.

3.3.2 Oil (%) in seed and oil yield

Oil content was observed to be 19.39% with single year application of Zn which increased to 19.44% and 19.61% with alternate and each vear application of Zn. With the increasing levels of Zn, Oil % was significantly increased over control. Minimum oil content 18.19% was found at control and maximum 20.17% was found at 10 kg Zn ha⁻¹. Similarly, oil yield 288.85 kg ha⁻¹ with alternate year application of Zn was also observed which was significant over single year, whereas at par with each year. With the increasing levels of Zn, oil yield was significantly increased up to 5 kg Zn ha⁻¹ then it was declined. Minimum oil yield 191.75 kg ha-1 was found at control and maximum 286.19 kg ha-1 was found at 5.0 kg Zn ha-1. The interaction effect was found to be statistically non-significant.

3.3.3 Carbohydrate (%)

The data showed that the alternate and each year Zn application increased carbohydrate content in seed as compared to single year (15.58%). However, single and each year application of Zn were statistically at par. The interaction effect was found to be statistically non-significant. The increasing levels of Zn significantly increased the Carbohydrate content in seed over control. Minimum carbohydrate content (13.39%) was found at control and maximum (16.10%) was found at 7.5 kg Zn ha⁻¹.

Zn application in soil enhanced the Zn concentration in the plant which is associated

with RNA and ribosome induction the result of which accelerates protein synthesis [25]. These results were also reported by Sonune et al., [25] Romani et al., [26] and Thakare et al., [27] Bairagi et al., [28] Dhanashree and Deshmukh [29] and Gaytri et al., [30].

3.4 Effect of Zn Application on Zinc Pools in Soil

It is clear from the results, that each year Zn application recorded the highest WS+EX-Zn,

Complexed-Zn, ASB-Zn, CSB-Zn, Residual-Zn and Total Zn fraction of 0.47, 1.10, 1.33, 4.03, 62.44 and 69.40 mg kg⁻¹, which was significantly higher than the single and alternate year Zn application. The each and alternate year Zn applications were at par in case of WS+EX-Zn, whereas case of complexed Zn periodicity was found to be non-significant. With single, alternate and each year Zn application, residual and total Zn were observed to be 57.42 and 63.40 mg kg⁻¹, 62.81 and 68.07 mg kg⁻¹ and 63.44 and 68.40 mg kg⁻¹, respectively.

Table 1. Effect of zinc application on yield attributes, yield and Zn uptake (g ha ⁻¹) by					
soybean(mean over the years)					

Treatments	Yield(t ha ⁻¹)		No. of pods plant ⁻¹	Seed test weight(g)	Zn uptake at harvest (g ha ⁻¹)	
	Seed	Stover		0 (0)	Seed	Stover
Periodicity						
Single Year application	1.15	1.97	66.00	7.72	58	26.26
Alternate Year	1.48	2.45	68.93	7.91	78.21	35.11
Each Year	1.29	2.39	67.40	7.89	68.93	34.46
SEm±	0.026	0.064	0.713	0.05	1.43	0.894
CD at 5%	0.101	0.252	NS	NS	5.624	3.514
Zn Levels (kg ha ⁻¹)						
0	1.05	1.93	59.89	7.58	52.21	23.77
2.5	1.24	1.92	67.56	7.81	64.06	25.81
5	1.43	2.54	71.44	8.08	74.6	35.15
7.5	1.41	2.49	70.00	7.89	74.95	37.22
10	1.40	2.46	68.33	7.85	76.1	37.76
SEm±	0.036	0.089	1.031	0.006	1.774	1.099
CD at 5%	0.102	0.254	2.944	0.018	5.069	3.139

NS-Non significant

Table 2. Effect of Zn application seed quality of soybean (mean over the years)

Treatments	Protein		Carbohydrate (%)	Oil			
	Protein (%)	Protein yield (kg ha ⁻¹)		Oil (%)	Oil yield (kg ha ⁻¹)		
Periodicity							
Single Year application	38.70	445.23	14.49	19.39	223.31		
Alternate Year	38.80	575.46	15.58	19.44	288.85		
Each Year	41.08	530.94	15.13	19.61	254.39		
SEm±	1.328	19.134	0.100	0.295	8.679		
CD at 5%	5.222	75.225	0.392	1.161	34.122		
Zn levels (kg ha ⁻¹)							
0	37.94	399.61	13.39	18.19	191.75		
2.5	39.41	488.03	14.64	19.17	237.30		
5	39.92	571.55	15.14	20.01	286.19		
7.5	40.06	562.31	16.10	19.86	279.11		
10	40.30	564.54	16.07	20.17	283.22		
SEm±	0.018	13.755	0.342	0.254	7.591		
CD at 5%	0.052	39.293	0.978	0.725	21.686		

Treatments	Zn fractions (mgkg ⁻¹)						
	WSEX-Zn	Comp-Zn	ASB-Zn	CSB-Zn	Residual	Total	
Periodicity							
Single Year	0.41	0.94	1.14	3.46	57.42	63.40	
application	(0.66)	(1.49)	(1.81)	(5.46)	(90.58)		
Alternate Year	0.44	0.97	1.14	3.68	62.81	68.07	
	(0.65)	(1.44)	(1.66)	(5.34)	(90.94)		
Each Year	0.47	1.10	1.33	4.03	62.44	69.40	
	(0.69)	(1.62)	(1.96)	(5.90)	(89.84)		
SEm±	0.008	0.091	0.030	0.051	0.269	0.449	
CD at 5%	0.032	0.358	NS	0.199	1.057	1.765	
Zn Levels (kg ha ⁻¹)							
0	0.39	0.86	1.09	3.30	58.66	64.33	
	(0.62)	(1.34)	(1.70)	(5.14)	(91.20)		
2.5	0.44	1.01	1.14	3.46	59.48	65.56	
	(0.68)	(1.55)	(1.75)	(5.28)	(90.74)		
5	0.44	1.03	1.28	3.65	60.68	67.11	
	(0.67)	(1.55)	(1.91)	(5.45)	(90.43)		
7.5	0.45	1.07	1.24	4.05	61.38	68.22	
	(0.67)	(1.58)	(1.82)	(5.95)	(89.98)		
10	0.47	1.04	1.29	4.15	62.58	69.56	
	(0.69)	(1.50)	(1.86)	(5.98)	(89.98)		
SEm±	0.010	0.049	0.050	0.071	0.193	0.373	
CD at 5%	0.028	0.140	0.144	0.203	0.550	1.065	

Table 3. Effect of Zn application on different fractions and percent contribution of various fractions of Zn to total soil Zn in post-harvest soil after soybean (mean over the years)

The data presented in Table 3 also indicate that the water soluble and exchangeable (WS+EX) Zn fraction were gently increased with increasing levels of Zn @ 2.5, 5.0, 7.5 and 10 kg ha-1 as compared to control. The maximum and significant WS+EX-Zn fraction of 0.47 mg kg⁻¹, in soil was recorded with 10 kg Zn ha-1 application over control. The application of 7.5 kg Zn ha-1 was at par with 10 kg Zn ha⁻¹. Similarly, application of 5.0 kg Zn ha⁻¹ was at par with 7.5 ha⁻¹. The minimum WS+EX-Zn, kg Zn Complexed-Zn, ASB-Zn, CSB-Zn, Residual-Zn and Total Zn fraction of 0.39, 0.86, 1.09, 3.50, 58.66 and 64.33 mg kg-1 were recorded under control. The Zn levels at 5, 7.5, and 10 kg Zn ha-1 were found at par among themselves for complexed fraction of Zn.

The highest ASB-Zn fraction 1.29 mg kg⁻¹ in soil was recorded with 10 kg Zn ha⁻¹ which was statistically superior as compared to all lower levels of Zn. The Zn levels at 5 and 7.5 kg ha⁻¹ were found at par and found to be significant over 2.5 kg Zn ha⁻¹. The content of Zn in CSB fraction was observed to be 3.30 mg kg⁻¹ at control and 3.46 mg kg⁻¹ at 2.5 kg Zn ha⁻¹ which significantly increased to 3.65, 4.05 and 4.15 mg kg⁻¹ with application of Zn @ 5.0, 7.5 and 10 kg Zn ha⁻¹, respectively. However 7.5 and 10 kg Zn

ha-1 application were statistically at par. Similarly, 2.5 and 5.0 treatments were at par. The interaction effect between Zn levels and periodicity was found to be statistically nonsignificant. It is fact from the data that more than 90 percent of the total Zn was found in residual fraction of Zn. While <10 percent was distributed in bio-available pool in the Vertisol. WS+EX-Zn, Complexed-Zn, ASB-Zn, CSB-Zn, fraction increased from 0.62 to 0.69, 1.34 to 1.50, 1.70 to 1.86 and 5.14 to 5.98 per cent respectively due to application of Zn over control.

The concentration and per cent contribution of Water soluble plus exchangeable zinc fraction (WSEX-Zn) to total Zn was the lowest among all the Zn fractions studied. Similar finding have been reported by Singh et al., [31]. Type of dominant clay minerals is the reason for variation in amount of residual Zn. This might be due to addition of Zn fertilizers and also due to occluded co-precipitating with hydrous oxide of or manganese, iron and form a principal matrix with abundant held zinc. Similar to WSEX -Zn, this fraction also contributed very less to total zinc and this could be due to medium organic matter content of the experimental soils. Similar finding were reported by Wijebandara et al., [32] and [33]. Based on result concluded that different fractions of soil Zn are in dynamic equilibrium with each other. Depletion of water soluble and exchangeable and complexed forms of Zn occurred with a build-up of organic, occluded and residual fractions of Zn occurred. The Similar results were also reported by Singh et al. [34], Safari et al. [35] and Ramzan et al. [36].

4. CONCLUSIONS

From the findings the it could be suggested that the level of 5.0 kg Zn ha-1 recorded significantly highest seed and stover yield (1.43 t ha-1 and 2.54 t ha-1) over control which was similar with that obtained with 5.0, 7.5 and 10 kg Zn ha⁻¹. The Zn concentration in seed increased significantly with increasing levels of Zn and highest at 10 kg Zn ha⁻¹. However, it was at par with 7.5 kg Zn ha⁻¹ ¹.The protein and oil content in seed was observed to be 41.08 and 19.44% and 38.80 and 19.61% with alternate and each year application of Zn, respectively. While <10 percent was distributed in bio- available pool in the Vertisol. WS+EX-Zn, Complexed-Zn, ASB-Zn, CSB-Zn, fraction increased from 0.62 to 0.69, 1.34 to 1.50, 1.70 to 1.86 and 5.14 to 5.98 per cent respectively, due to application of Zn over control. More than 90 percent of the total Zn was found in residual fraction of Zn. The pH, EC and OC of soil samples indicated non-significant variation. Zn in soil was highest with 5.0 kg Zn ha-1. Finally it cloud be concluded the alternate year application of 5 kg Zn ha-1 (25 kg ZnSO₄.7H₂O) gave the optimum yield and good quality produce as well as bio-available pools of zinc in soil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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