



# **Biofortification of Potato Tubers with Zinc Fertilizer Through Soil**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors SA and MAR designed the study, author SA performed statistical analyses, wrote the protocol, wrote the first draft of the manuscript. Author MIH edited the manuscript. All authors read and approved the final manuscript.*

## **Article Information**

DOI: <https://doi.org/10.9734/aprj/2025/v13i1290>

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/129162>

**Original Research Article**

**Received: 30/10/2024**

**Accepted: 01/01/2025**

**Published: 08/01/2025**

## **ABSTRACT**

Zinc deficiency is a well-documented problem in food crops, causing decreased crop yield and nutritional quality. Generally, the regions in the world with Zn-deficient soils are also characterized by widespread Zn deficiency in humans. Nearly 25% of the world's population is considered to be zinc (Zn)-deficient. An estimated 44.6% of preschool-age children are at risk of Zn deficiency, while 57% of non-pregnant and non-lactating women are deficient in Zn in Bangladesh. The key reasons for such deficiency are an intensification of cropping systems and the adoption of high-yielding cultivars of crops. Plant breeding approach (e.g., genetic biofortification) is a long-term processes requiring substantial effort and resources. It is essential to have a short-term approach to improve Zn concentration in tubers. Application of Zn fertilizer (e.g., agronomic biofortification) offers a rapid solution to the problem and represents a useful complementary approach to ongoing breeding

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**Cite as:** Ahammed, S., M. A. Rahim, and M. I. Hossain. 2025. "Biofortification of Potato Tubers With Zinc Fertilizer Through Soil". *Asian Plant Research Journal* 13 (1):23-36. <https://doi.org/10.9734/aprj/2025/v13i1290>.

programs. The experiment was set up in a two-way factorial randomized complete block design with three replications. Soil application of Zn resulted in a significant increase of tuber Zn concentration with an increase of tuber yield of potato under study. Tuber yield 17-23% and tuber Zn concentration 56-116% increased over control as influenced by added Zn in soil @ 8 kg ha<sup>-1</sup> and this rate also reached an average of 1.92-fold tuber Zn increase of the varieties. Zinc biofortification strategy appears to be essential in keeping sufficient amounts of available Zn in soil solution and maintaining adequate Zn transport to the seeds during the reproductive growth stage. Enrichment of potatoes with high bioavailable Zn is suggested as a way to generate significant health benefits for a large number of susceptible people across the world. Finally, agronomic biofortification would be a very attractive, quick and useful strategy in mitigating widespread Zn-driven malnutrition-related health problems globally and effectively.

**Keywords:** Malnutrition; bioavailability; BARI (Bangladesh Agricultural Research Institute); Au (potato).

## 1. INTRODUCTION

“Micronutrient malnutrition known as hidden hunger is the most widespread nutritional problem all over the world, especially for women, infants, and children” (Stein, 2010). “Micronutrient deficiency is a global health concern” (Praharaj, et al., 2021, Gold, et al., 2022). The aim of this study was to investigate the concept that the potato (*Solanum tuberosum* L.) can agronomically be biofortified with zinc (Zn) fertilization. Nearly 25% of the world's population is considered to be zinc (Zn)-deficient (Allai, et al., 2022, Poudel, et al., 2023), and approximately 30% of children in the world have stunted growth mainly due to Zn deficiency (Brown, 2007). Zinc is one of the essential nutrients for animal as well as for plants (Gupta, et al., 2020). “In Bangladesh, over 41% of children aged below five years are stunted, while an estimated 44% children of the same age group are at risk of zinc deficiency” (NIPORT, 2013; Rahman, et al., 2016). Over two billion people across the world suffer from micronutrient malnutrition (Praharaj, et al., 2021). “Food insecurity, imbalanced diet, consumption of food grains with poor nutritional quality and, lack of dietary diversity, etc., negatively affect human health” (Gundersen & Ziliak, 2015). Biofortification of major cereals (e.g. rice, wheat and maize), tuber crops (e.g. potato, cassava), edible oil (soyabean) and fruits (banana, watermelon) can supply the required amount of dietary zinc (Bhardwaj, et al., 2022).

“It is estimated that about one-third of the cultivated soils globally contain low amounts of available Zn, resulting in impaired crop production and also the low nutritional quality of the harvested grains” (Cakmak, 2008); “nearly 50% of cereal-growing areas in the world have been found deficient in Zn” (Cakmak, 2008);

“more than 50% of the Asian soils are Zn deficient” (Singh & Gupta, 2005). In humans, Zn deficiency can cause reduced immune and reproductive function (Younas, et al., 2023), impaired brain function (Hambidge, 2000), physical retardation (Prasad, 2015) and stunted growth (Kiran, et al., 2022), which is now a significant health concern in developing countries (Roohani, et al., 2013).

Biofortification aims to increase nutrient levels in crops during plant growth. “The food's micronutrient level need to rise for improved human and animal health. Preliminary studies indicate that Zn enrichment of potato tubers is possible through Zn fertilization, and the magnitude depends on the crops and varieties” (Mengist, et al., 2021; Haynes, et al., 2012).

Potato (*Solanum tuberosum* L.) is most important and common vegetable in Bangladesh. “Next to rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), potato is the third major food crop in Bangladesh. It also ranks just behind the cereals rice and wheat and achieved 3rd position among the food crops worldwide” (Birch, et al., 2012). “More than a billion people eat potatoes regularly” (Kromann, et al., 2017); “hence, one of the main sources of Zn to humans” (Mengist, et al., 2018a; Subramanian, 2012; White, et al., 2012).

Enrichment of potatoes with high bioavailable Zn is suggested as a way to generate significant health benefits for a large number of susceptible people, especially in Bangladesh as well as Zn-deficient areas in the world (Olsen & Sommers, 1982; White & Broadley, 2009). “Agronomic biofortification can be an economically sustainable and practically acceptable solution to solve Zn deficiency in potatoes” (Mahmud, et al., 2021; Bhatt, et al., 2020; Baghla, et al., 2019);

Sarkar, et al., 2018). Zinc loading in potato through soil-applied Zn resulted 56–116% tuber Zn concentrations increase over unfertilized control under study.

## 2. MATERIALS AND METHODS

### 2.1 Planting Materials and Variety

The seed tubers were collected from Bangladesh Agricultural Research Institute (BARI), Debiganj, Panchagor, Bangladesh and Bangladesh Agricultural Development Corporation (BADC), Jamalpur, Bangladesh. The seed tubers (grade A, i.e., 28-41 mm in diameter) were kept under diffused light conditions for sprouting. Well-sprouted seed tubers were planted in the experimental field. A few seed tubers were cut longitudinally into 2-3 pieces; each piece had at least two eyes. The weight of each piece of tuber was about 15-16 g. The seed tubers were treated with Autostin (soaked in @ 2 g L<sup>-1</sup> fresh water). The cuttings were exposed to the sunlight for a few minutes. The cut tubers were planted in the field just after removing the surface moisture. The experiment involved six varieties: BARI Alu-7, BARI Alu-13, BARI Alu-25, BARI Alu-53, BARI Alu-73 and BARI Alu-77. Among the varieties, BARI Alu-53 and BARI Alu-77 have late maturity, dense foliar cover with high yield potential, and a dense root system, and BARI Alu-53 has the densest foliar cover among the varieties. The other four are the comparatively early-maturing varieties with medium yield potential and moderate foliar cover.

### 2.2 Experimental Design and Treatments

The experiment was set up in a medium-high land at Horticulture Farm, Bangladesh Agricultural University (BAU), Mymensingh (24°42'56.04"N and 90°25'31.01"E, altitude=14 m), Bangladesh. The soil in the experimental field was a non-calcareous Dark Grey silt-loam.

In order to find out the effect of zinc on 6 potato genotypes, and a field experiment was conducted using a Randomized Complete Block Design (RCBD) with four different treatments and three replications during 2019-20. Next year in 2020-21, the field experiment was repeated to validate the results.

There were three blocks representing three replications. Each block contained 24-unit plots to accommodate treatment combinations (6 varieties x 4 treatments). Therefore, a total of 72 (24 x 3) unit plots were maintained in the experiments. The size of each unit plot was 3.6

m<sup>2</sup> (1.5 m x 2.4 m). Potato seed tubers were planted, maintaining a row-to-row distance of 60 cm and 25 cm between plants in a row. Each unit plot had 4 rows, where 6 seeds were planted in each row, resulting in 24 plants in each unit plot.

The experiment included the following four fertilizer treatments involving Zn0 (no Zn fertilizer), Zn1=4 kg Zn ha<sup>-1</sup>, Zn2=8 kg Zn ha<sup>-1</sup>, Zn3=12 kg Zn ha<sup>-1</sup> applied as zinc sulphate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) to soil at planting. The experiment was designed to evaluate Zn applications in soil on concentration of Zn in harvested tubers.

### 2.3 Determination of Dry Matter and Mineral Concentrations

Potato tubers (peeled) were washed in running water followed by distilled water, prepared, oven-dried at 70<sup>o</sup> C, dry weight (dw) determined, ground to powder, and analytical samples taken of 0.5 g with 5.0 ml Trace Element Grade (TEG) concentrated nitric acid (HNO<sub>3</sub>) for pre-digestion overnight and digested with 3.0 ml of 30% (v/v) hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at 140<sup>o</sup> C following an adaptation of the methods described by Subramanian et al. (2011); the current standard method used by Agri varsity Humboldt Soil Testing Laboratory for Management of Soils and Water, Department of Soil Science, BAU, Mymensingh, Bangladesh. Finally, the acid-digested plant samples were analyzed in the Atomic Absorption Spectrophotometer (AAS) for Zn content (µg g<sup>-1</sup>) in Soil Resources Development Institute (SRDI)'s Laboratory for Management of Soils and Water in Dhaka, Bangladesh.

### 2.4 Statistical Analysis

Plant parameters (yield and yield components) and tuber Zn concentration data were subjected to statistical analysis through the statistical program Minitab 17 (Minitab Inc., State College, PA, USA) following the basic principles as outlined by Gomez and Gomez (1984). When significant differences were found in ANOVA, means were compared using Tukey's test at  $P \leq .05$ . Multiple treatment comparisons were made using orthogonal contrasts. The analysis of variance (General Linear Model Procedure) and Tukey's pairwise comparison test by Duncan's Multiple Range Test (DMRT) at 5% level of probability, according to Gomez & Gomez, (1984). Atypical data were treated as missing values in the statistical analysis. Physical and chemical properties of soil of the experimental fields are presented in Table 1.

**Table 1. Physical and chemical properties of soil used for the study**

Soil properties	
Soil texture	Silt loam
Sand	22.89%
Silt	73.23%
Clay	3.65%
pH (1:2.5 soil -water)	6.37
Total Nitrogen (%)	0.11
Available phosphorus (mg kg <sup>-1</sup> ) (Olsen extract)	9.52
Exchangeable potassium (meq/100 g soil)	0.11
Available sulphur (mg kg <sup>-1</sup> )	6.87
Total Zn (mg kg <sup>-1</sup> )	54.36
Available Zn (mg kg <sup>-1</sup> ) (Olsen extract)	0.81
Organic matter (%)	1.39

### 3. RESULTS

Biofortification is a biological process by which the nutritional quality of food crops is improved through conventional plant breeding, modern biotechnology, or agronomic management practices. The aim of this research was to enhance Zn concentrations of potato tubers with increasing or without affecting crop yield by using 6 varieties accompanied by the application of respective fertilizers in soil. The current experiments showed a statistically significant increase of tuber Zn concentration with an increase of tuber yield of potato, but excessive application reduced both tuber yield and tuber Zn concentration.

#### 3.1 Genotypic Effects

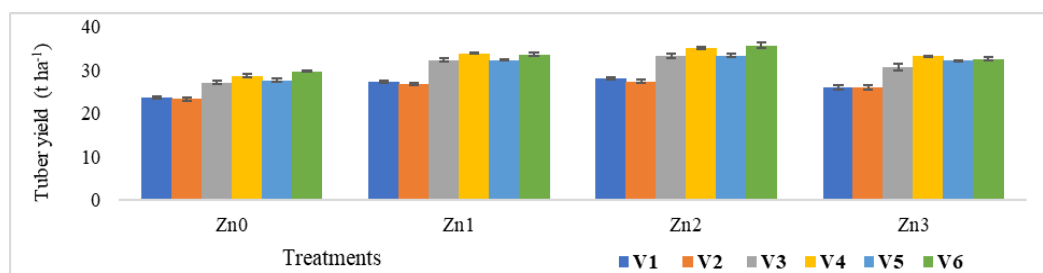
The yield of potato tuber varied with varieties which can be attributed to differences in genetic make-up. Genotypes had a significant effect on tuber yield of potato (data not shown). The tuber yield of trial average varied between 25.88 and 32.92 t ha<sup>-1</sup> (Table 2) which illustrates a significant varietal effect. In Bangladesh potato cannot be planted year-round; but in winter (November to February-March), when the

average temperature remains 18-22<sup>o</sup> C at the experimental field area. Experiments were intensively managed with irrigation.

#### 3.2 Agronomic Zn-Biofortification of Potato with Soil Applied Zn Fertilizer

Application of Zn fertilizer in soil had significant effects on tuber yield of potato. Due to Zn application in soil, of the tested genotypes, as observed the 2019-20 and 2020-21 trials (not shown) including trial average results, tuber yield of potato increased in Zn level from 4-8 kg Zn ha<sup>-1</sup> (Zn1-Zn2 treatment) but decreased with the increase in Zn level at 12 kg Zn ha<sup>-1</sup> (Zn3 treatment) for six potato genotypes. The highest tuber yield was 35.73 t ha<sup>-1</sup> as noted in BARI Alu-77, followed by 35.10 t ha<sup>-1</sup> as found in BARI Alu-53 (Table 3 and Fig. 1).

The individual ANOVAs of the effect of Zn fertilizer rates on tuber yield revealed a significant effect on soil Zn application ( $P = .000$ ). The Tukey ranking ( $P < .05$ ) indicated a significant increase of the tuber yield between the control and the lowest Zn rate of soil Zn rate of all the potato varieties (Table 3). Combined



**Fig. 1. Effects of Zn treatments in soil on tuber yield (t ha<sup>-1</sup>) of six varieties of potato at Horticulture Farm, BAU, Mymensingh, Bangladesh ((results are the average of two trials: 2019-20 and 2020-21)**

**Table 2. Varietal effect on marketable yield (t ha<sup>-1</sup>) of potato tubers of Zn application in soil of trial 1, trial 2 and trial average**

Variety	Yield (t ha <sup>-1</sup> )		
	Trial 1 (2019-20)	Trial 2 (2020-21)	Trial average
V1 (BARI Alu-7)	27.60±0.58 d	24.97±0.50 c	26.29±0.53 c
V2 (BARI Alu-13)	26.48±0.67 e	25.28±0.34 c	25.88±0.49 c
V3 (BARI Alu-25)	32.72±0.81 c	29.01±0.69 b	30.86±0.75 b
V4 (BARI Alu-53)	33.81±0.74 ab	31.74±0.78 a	32.76±0.74 a
V5 (BARI Alu-73)	33.04±0.77 bc	29.81±0.65 b	31.43±0.70 b
V6 (BARI Alu-77)	34.44±0.71 a	31.40±0.68 a	32.92±0.69 a
Max	34.44	31.74	32.92
Min	26.48	24.97	25.88
Mean	31.35	28.70	30.02
LSD <sub>0.05</sub>	0.62 *	0.68 *	0.61 *
LSD <sub>0.01</sub>	0.83 **	0.90 **	0.82 **
Level of significance	**	**	**
<i>P</i> -value	<0.001	<0.001	<0.001

\*\* = Significant at 1% level of probability;

Values in the same column followed by same letter are not significantly different according to Tukey's test (*P*<.05)

**Table 3. Interaction effects of Zn application in soil on the tuber yield (t ha<sup>-1</sup>) of six varieties of potato at Horticulture Farm, BAU, Mymensingh, Bangladesh ((results are the average of two trials:2019-20, 2020-21)**

Variety	Treatment			
	Zn0	Zn1	Zn2	Zn3
V1 (BARI Alu-7)	23.62 i	27.29 gh	28.12 f-h	26.11 h
V2 (BARI Alu-13)	23.35 i	26.76 gh	27.40 f-h	26.01 h
V3 (BARI Alu-25)	27.08 gh	32.36 cd	33.29 bc	30.71 de
V4 (BARI Alu-53)	28.75 e-g	33.95 a-c	35.10 ab	33.23 bc
V5 (BARI Alu-73)	27.66 f-h	32.37 cd	33.40 a-c	32.28 cd
V6 (BARI Alu-77)	29.68 ef	33.64 a-c	35.73 a	32.62 cd
<b>Max</b>	<b>29.68</b>	<b>33.95</b>	<b>35.73</b>	<b>33.23</b>
<b>Min</b>	<b>23.35</b>	<b>26.76</b>	<b>27.40</b>	<b>26.01</b>
<b>Mean</b>	<b>26.69</b>	<b>31.06</b>	<b>32.17</b>	<b>30.16</b>

Mean values with the same letters are not significantly different based on ANOVA followed by a Tukey's test at *P*<.05

[Zn0=no Zn fertilization, Zn1=@ 4 kg Zn ha<sup>-1</sup>, Zn2=@ 8 kg Zn ha<sup>-1</sup> and Zn3=@ 12 kg Zn ha<sup>-1</sup> as applied in soil before planting]

ANOVAs revealed no significant effect on tuber yield (*P*=.116). Interestingly, no negative effects on yield of the high Zn applications were seen with the edaphic conditions and fertilizer types of this study.

A highly significant effect of Zn fertilizer treatments on tuber Zn concentration of peeled potato tubers was detected in the average results (Table 4) of field trial 1 in 2019-20 & trial 2 in 2020-21 when Zn fertilizer was applied in soil.

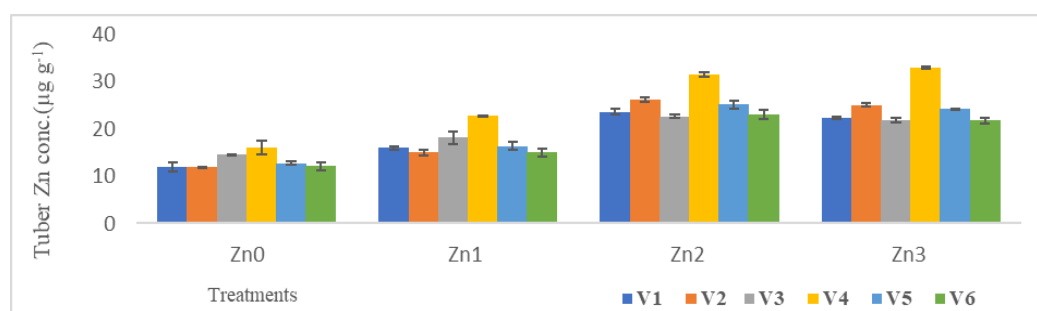
Zn application in soil trial in 2019-20 which was repeated next year in 2020-21 for validation of

results (data not shown). The trial average result showed a gradual increase in tuber Zn concentrations with increased application rates of Zn in soil (Table 4). The results also showed that the tuber Zn concentration increased with increasing Zn dose up to 8 kg ha<sup>-1</sup> (Zn2 treatment) followed by a decrease in tuber Zn concentration at 12 kg Zn ha<sup>-1</sup> (Zn3 treatment) level for five varieties among six (Table 4 and Fig. 2). 56–116% tuber Zn concentrations increased over control due to added Zn in soil, and the increment of tuber Zn concentration due to added Zn @ 8 kg ha<sup>-1</sup> in soil was found highest (31.55 µg g<sup>-1</sup>) in the BARI Alu-53 variety.

**Table 4. Interaction effects of Zn treatments in soil on Zn concentration ( $\mu\text{g g}^{-1}$ ) of tubers of six genotypes of potato (results are the average of two trials: 2019-20 and 2020-21)**

Variety	Treatment			
	Zn0	Zn1	Zn2	Zn3
V1 (BARI Alu-7)	11.93 g	15.96 ef	23.66 bc	22.34 c
V2 (BARI Alu-13)	12.12 g	15.02 g	26.21 b	25.08 bc
V3 (BARI Alu-25)	14.51 e-g	18.12 de	22.65 bc	21.88 cd
V4 (BARI Alu-53)	15.96 ef	22.76 bc	31.55 a	33.02 a
V5 (BARI Alu-73)	12.69 fg	16.38 de	25.15 bc	24.18 bc
V6 (BARI Alu-77)	12.12 g	15.00 e-g	23.07 bc	21.74 cd
<b>Max</b>	<b>15.96</b>	<b>22.76</b>	<b>31.55</b>	<b>33.02</b>
<b>Min</b>	<b>11.93</b>	<b>15.00</b>	<b>22.65</b>	<b>21.74</b>
<b>Mean</b>	<b>13.22</b>	<b>17.21</b>	<b>25.38</b>	<b>24.71</b>

( $P < .001$ ); Values in the same column followed by the same letter are not significantly different according to Tukey's test ( $P < .05$ )



**Fig. 2. Effects of Zn treatments in soil on Zn concentration ( $\mu\text{g g}^{-1}$ ) of tubers of six genotypes of potato (results are the average of two trials: 2019-20 and 2020-21)**

**Table 5. Varietal effects on tuber zinc concentration ( $\mu\text{g g}^{-1}$ ) of potato by applying Zn in soil of trial 1, trial 2 and trial average**

Variety	Zn concentration ( $\mu\text{g g}^{-1}$ )		
	Trial 1 (2019-20)	Trial 2 (2020-21)	Trial average
V1 (BARI Alu-7)	17.71±1.40 b	19.24±1.59 b	18.47±1.46 bc
V2 (BARI Alu-13)	19.12±1.93 b	20.00±1.88 b	19.56±1.88 b
V3 (BARI Alu-25)	18.99±1.09 b	19.59±1.08 b	19.29±1.03 bc
V4 (BARI Alu-53)	24.78±2.09 a	26.87±2.17 a	25.82±2.11 a
V5 (BARI Alu-73)	18.57±1.58 b	20.63±1.64 b	19.60±1.60 b
V6 (BARI Alu-77)	17.23±1.40 b	18.74±1.45 b	17.98±1.42 c
Max	24.78	26.87	25.82
Min	17.23	18.74	17.98
Mean	19.40	20.85	20.12
LSD <sub>0.05</sub>	1.30 **	1.30 **	0.99 **
LSD <sub>0.01</sub>	1.74 **	1.73 **	1.32 **
Level of significance	**	**	**
P-value	0.000	0.000	0.000

Means followed by same letter in a column do not differ significantly at 5% level of probability; \*\* = Significant at 1% level of probability

A highly significant effect of Zn fertilizer rate was seen on tuber Zn concentration in the year average result (ANOVA,  $P$  value  $< .005$  for all comparisons). Soil applied Zn significantly increased tuber Zn concentrations in 6 potato cultivars (Table 4). The highest

performer treatment (Zn2 treatment) increased the Zn concentration in tuber flesh 1.98, 2.16, 1.56, 1.97, 1.98, 1.90-fold over control in BARI Alu-7, BARI Alu-13, BARI Alu-25, BARI Alu-53, BARI Alu-73, BARI Alu-77 respectively (Table 4).

The potato genotypes that showed high tuber Zn concentrations in the absence of Zn fertilization also showed in Table 5 and even higher tuber Zn concentrations following Zn fertilization (Table 4).

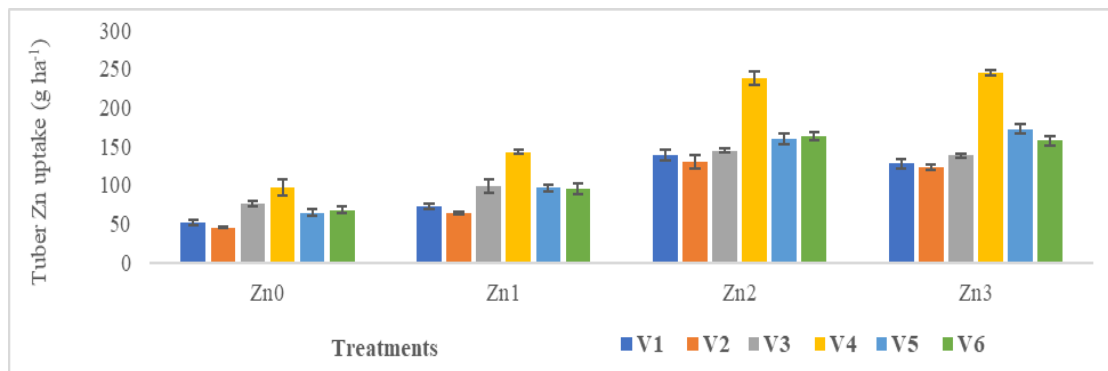
A highly significant effect of Zn fertilizer treatments was found Zn uptake by tubers from soil Zn application trial (ANOVA,  $P$  value < .005 for all comparisons). Zn uptake by tubers significantly increased with the progressive increase in Zn application levels from 0 to 12 kg

$\text{ha}^{-1}$  Zn application (Zn0 to Zn3 treatment) in two varieties of BARI Alu-53 & BARI Alu-73 among six; on the other hand, Zn uptake by tubers significantly increased with the progressive increase in Zn application levels from 0 to 8 kg  $\text{ha}^{-1}$  Zn application (Zn0 to Zn2 treatment) but decreased with the increase in Zn level 12 kg  $\text{ha}^{-1}$  Zn application (Zn3 treatment) in other four varieties of potato BARI Alu-7, BARI Alu-13, BARI Alu-25, BARI Alu-77 (Table 6 and Fig. 3).

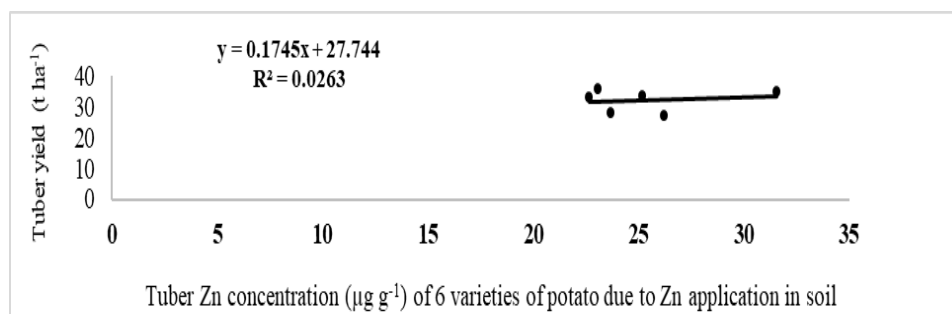
**Table 6. Interaction effects of Zn treatments in soil on Zn uptake ( $\text{g ha}^{-1}$ ) by tubers of six genotypes of potato (results are the average of two trials: 2019-20 and 2020-21)**

Variety	Treatment			
	Zn0	Zn1	Zn2	Zn3
V1 (BARI Alu-7)	52.35 k	72.20 i-k	138.12 c-f	128.23 e-g
V2 (BARI Alu-13)	45.20 k	63.92 jk	129.99 d-f	122.86 f-h
V3 (BARI Alu-25)	75.77 i-k	98.73 g-i	144.90 b-f	138.31 c-f
V4 (BARI Alu-53)	96.25 hi	142.89 b-f	238.40 a	244.97 a
V5 (BARI Alu-73)	64.62 jk	96.51 hi	160.33 b-d	172.10 b
V6 (BARI Alu-77)	68.18 i-k	94.87 h-j	163.11 bc	157.23 b-e
<b>Max</b>	<b>96.25</b>	<b>142.89</b>	<b>238.40</b>	<b>244.97</b>
<b>Min</b>	<b>45.20</b>	<b>63.92</b>	<b>129.99</b>	<b>122.86</b>
<b>Mean</b>	<b>67.06</b>	<b>94.85</b>	<b>162.48</b>	<b>160.62</b>

Mean values with the same letters are not significantly different based on ANOVA followed by a Tukey's test at  $P < .05$



**Fig. 3. Interaction effects of Zn treatments in soil on Zn uptake ( $\text{g ha}^{-1}$ ) by tubers of six genotypes of potato (results are the average of two trials: 2019-20 and 2020-21)**



**Fig. 4. Relationship between tuber yield and tuber Zn concentrations ( $\mu\text{g g}^{-1}$ ) for Zn added effects over control treatment in different varieties of potato**



### 3.3 Correlation Analysis

A comparison of the mean Zn concentrations with the mean tuber yields in the field trials with Zn fertilization in soil reveal that the micronutrient concentrations in tubers have a positive correlation with tuber yield (Fig. 4).

Again, a comparison of the mean Zn uptake by tubers with the mean tuber yields in the field trials with Zn application in soil show that the micronutrient uptake by tubers have a positive correlation with tuber yield (Fig. 5).

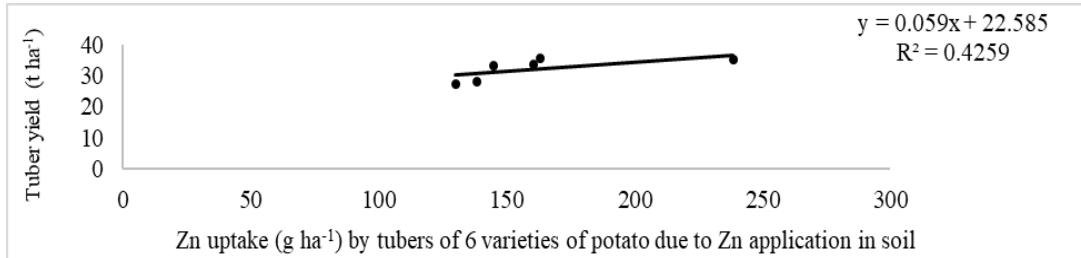


Fig. 5. Relationship between tuber yield and Zn uptake by tubers (g ha<sup>-1</sup>) for Zn added effects over control treatment in different varieties of potato



Pic 1. Zinc content estimation in Lab

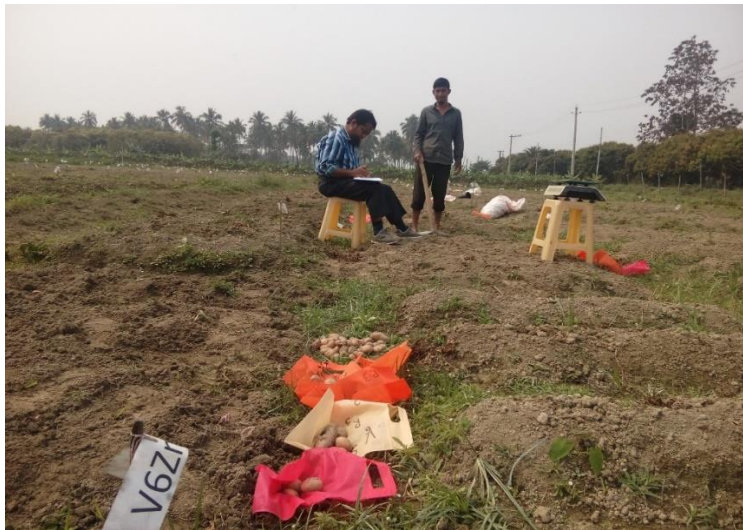


Pic 2. Data collection





**Pic 3. Plant height estimation**



**Pic 4. Tuber weight measurement**



**Pic 5. Experimental Field**

#### 4. DISCUSSION

The field trials with Zn fertilizers showed a significant effect of the Zn fertilizer treatments on tuber Zn concentrations (Table 4). Soil chemical factors such as low pH in the BAU soils, and potato genotypic factors, may be some of the reasons for the good effectiveness of the soil Zn treatments in these experiments. To elucidate the efficacy and validation of soil-applied Zn rates a repetition of field experiments were done. These experiments, conducted at the same location of previous field experiments, resulted in a 1.93-fold and 1.87-fold (average value) tuber Zn increase over control due to added zinc in soil @ 8 kg ha<sup>-1</sup> & 12 kg ha<sup>-1</sup> respectively (Table 4).

The present results are comparable to many works in the past. Kromann, et al., (2017) conducted a study to evaluate a fertilizer approach's potential role in increasing Zn concentrations in Andean potato cultivars through a series of investigations in Ecuador. The results confirmed that Andean potato cultivars could be agronomically Zn-biofortified with soil applied Zn fertilizers. Zn rates @ 8 kg ha<sup>-1</sup> applied in soil reached a 1.91-fold tuber Zn increase in the field trials. The results conform with those of Phattarakul, et al., 2012; Zou, et al., (2012) reported that soil Zn application is effective in Zn biofortification of food crops.

White, et al., 2012 and Murmu, et al., 2014 also reported from their study that Zn loading in potato through soil-applied Zn, increases Zn concentration in potato tuber up to 3-4 times (30-40 mg Zn kg<sup>-1</sup> of dry matter) which is quite higher than most of the commonly known fruit crops. These findings are in agreement with Mahmud, et al., (2021) who reported from their study that soil application of Zn resulted in a significant increase of tuber yield with an increase of Zn concentration in potato tubers.

“Soil-applied Zn is, however, limited depending on soil properties and inherent Zn status in the upper soil layer” (Sawicka, et al., 2016). The efficacy of soil-applied fertilizer largely depends on the soil environment (pH, moisture content, soil organic matter, presence of antagonist nutrients etc.) and the ability of plant to successfully absorb the nutrients.

The potato varieties that showed high tuber Zn concentrations in the absence of Zn fertilization (Table 5) also showed correspondingly higher tuber Zn concentrations following soil applied Zn

(Table 4). Nevertheless, Zn soil uptake and translocation to haulms and tubers may vary significantly among varieties. “Many andigenum-type cultivars have different root system architecture compared to tuberosum-type cultivars” (Wishart, et al., 2013).

“Zinc sulphate has been widely used to biofortify crops with Zn” (White, & Broadley 2011; Velu, et al., 2014). The various forms of Zn fertilizer and ways of applying them to the potato crop may influence the efficiency of uptake and translocation to tubers differently. In our trials we used zinc sulphate fertilizers because it is commonly used and readily available in Bangladesh, with the objective to study fertilizer effects more likely to be taken up and translocated to tubers. For practical recommendations on agronomic biofortification of potato with Zn additional studies are needed to define economic optimal compounds, application methods, and rates.

Considering the low soil Zn level in Bangladesh soils that appear to be a major factor causing low human Zn intake, agronomic Zn biofortification of potato may prove to be an important approach to improve the critical human Zn status in Bangladesh.

The tuber yields positively responded to Zn fertilization applied in soil over the six varieties of potato. The highest tuber yield (35.73 t ha<sup>-1</sup>) was recorded from the Zn dose of 8 kg Zn ha<sup>-1</sup> (Zn2 treatment) obtained by BARI Alu-77, followed by BARI Alu-53 (35.10 t ha<sup>-1</sup>). Interestingly, the tuber yields further declined to some extent in the next higher Zn dose of 12 kg Zn ha<sup>-1</sup> (Zn3 treatment).

The present results agree with Banerjee, et al., (2016) who conducted a field experiment at Kakdwip, South 24-Parganas, West Bengal, in India during 2013-14 and 2014-15 to assess the Zn requirements of potatoes on a new alluvial soil (Entisol). They found an increase in tuber yield with increasing Zn dose up to 4.5 kg ha<sup>-1</sup> in soil. Sarkar, et al., (2018) observed that Zn fertilization produced higher tuber yield of potatoes and higher tuber Zn concentration. Brahmachari, et al., (2010) also recorded a 9.2% yield increment for using Zn-fortified seeds. Mahmud, et al., (2021) reported that soil application of Zn resulted in a significant increase in tuber yield with an increase in Zn concentration in potatoes. “The same result applies to a study where the potato tuber seeds

collected from the Zn-treated plots, gave better results regarding tuber yield and tuber Zn concentrations. It is particularly important for Zn-deficient soil” (Cakmak, 2010).

Dwivedi and Dwivedi (1992) showed that 10 kg ZnSO<sub>4</sub> ha<sup>-1</sup> was adequate to increase potato tuber yield and starch content. They concluded that starch content in potato tubers were affected not only by Zn rate but also by the method of Zn application. In their study, starch content of tuber was significantly affected by soil application (10 kg ZnSO<sub>4</sub> ha<sup>-1</sup>) as well as seed soaking with Zn (R<sup>2</sup>=0.602). Kumar, et al., (2008a) opined that greater accumulation of starch depends on the higher rate of photosynthesis, better translocation of photosynthates from leaves to tubers and subsequent conversion to starch. Therefore, increased starch accumulation in tubers might be due to higher rate of photosynthesis with zinc application.

The increase in tuber yield due to Zn fertilization might be because Zn played a vital role in the biosynthesis of the IAA (Indole-3 Acetic Acid) and initiation of primordia for reproductive parts a result of the favorable effect of zinc on the metabolic reactions within the plants. However, such an investigation was not done in the present study. “The increased tuber yield of potato might be attributed to the beneficial effect on tuberization as a result of Zn application (Mondal, et al., 2015) and Zn content in tubers” (Singh, et al., 2009; Singh, et al., 2010). “The reason is zinc plays a key role in plant metabolism, particularly in auxin synthesis, which is an essentially required for the growth and development of a crop” (Sarkar, et al., 2018).

Zinc activates the glutamic dehydrogenase enzyme, synthesizing of RNA and DNA enhancing gliadin and glutenin contents, which are the main protein components of gluten accumulated in the later stage of tuber forming. The present study hints that the scope exists to enhance tuber Zn concentration by applying Zn fertilizer. Lone, et al., (2017) reported enhanced tuber Zn concentration due to increasing soil Zn supplied when sufficient Zn was available to the plants. This suggests a proper combination of N and Zn applications.

Soil applied Zn significantly increased Zn uptake by tubers of 6 potato varieties (Table 6), which shows that on an average, Zn uptake by tubers increased 2.46-fold and 2.42-fold over control (no Zn fertilizer) due to Zn application in soil @ 8 kg

ha<sup>-1</sup>, 12 kg ha<sup>-1</sup> respectively. Similar findings were described by Banerjee et al., (2016) that zinc uptake by tubers, haulm, and whole plant significantly increased with the progressive increase in Zn application levels from 0 to 6 kg ha<sup>-1</sup> Zn application in soil.

The tuber Zn concentrations of commonly used potato varieties need to be improved to fulfil the Zn requirement for the people in Bangladesh. “Zinc concentration in potato tubers can be enriched by: i) biofortification with Zn fertilizers” (White, et al., 2017; Kromann, et al., 2017), ii) manipulating Zn transporters and ligands in potato plants (Mengist, et al., 2021; Ricachenevsky, et al., 2013) and iii) efficient germplasm screening for higher bioavailable Zn (Haynes, et al., 2012). All these methods depend on fertilizer or the soil, or both as the source of Zn to produce Zn- enriched tubers. “Soil-supplied Zn is, however, limited depending on soil properties and inherent Zn status in the upper soil layer” (Sawicka, et al., 2016). “Zn concentration in any crop grown in Zn-sufficient soils also increases significantly with increased Zn application” (Zhang, et al., 2012). Systematic survey and mapping of plant-available soil Zn concentrations across the Asian countries and other areas with apparent low soil Zn levels would aid the identification of areas with high potential for impact from Zn biofortification.

## 5. CONCLUSION

Plants are the beginning of the food chain and make loads of nutrients for other organisms to eat. Since zinc deficiency is highly prevalent in Bangladesh and principally related to inadequate quality of diet, micronutrient management (especially Zn) has received greater attention in potato cultivation practice to combat widespread Zn deficiency. Potato responds well to the applied Zn fertilizer (4 - 8 kg Zn ha<sup>-1</sup>). Therefore, improving the Zn uptake from soil and increasing its movement and bioavailability in the edible parts of the potato plant will benefit human and animal nutrition. Zinc plays an important role in the growth, productivity, and post-harvest quality of potato. Besides, Zn biofortification with potato tubers will be necessary in the future to fully understand the number of nutrients in the soil plant ecosystem and holds great effects for malnutrition problems in human health, and it can be a potential option for mitigating widespread Zn-driven malnutrition for the world population.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## ACKNOWLEDGEMENTS

The authors are thankful to the Bangladesh Agricultural University, Bangladesh Water Development Board and Ministry of Water Resources, Government of Bangladesh for providing facilities to carry out the research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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