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Research Article

Assessment of zinc phyto availability by two cotton genotypes amending Zn-sulfate under in-situ condition

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Abstract

The study was carried out at Agriculture Research Institute, Jaffarabad at Usta Mohammed Balochistan. A wire house pot experiment was performed in Completely Randomized Design (CRD) with three replications. The aimed to assess efficacy of Zinc sulfate ($ZnSO_4.7H_2O$), as soil amendment on plant dry biomass, phyto availability and macronutrient uptake by two cotton genotypes (NIAB-78 and Bt-802) under in-situ condition. The top soil was treated with different concentration of Zinc (Zn) viz: Ck, 0.01, 0.02, 0.04, 0.08, and 0.16 mg L^{-1}(w/v) hydrated $ZnSO4{\cdot}7H_2O$. The results indicated thatZn-0.04 mg L^{-1} application rate maximally increased the shoot dry biomass by 31.64% for NIAB-78 and35.82% for BT-802 respectively. Meanwhile, as compared to control the Zn concentration, uptake in shoot biomass and Zn utilization efficiency were increased with addition of amendment rates. However NPK concentration in shoot biomass was observed higher in NIAB-78 than BT-802 genotype. Such research should be focused on soil type, Zn-fractions and its mechanism under field condition and can improve soil fertility with essential plant nutrients and could be fruitful results in future sustainable agriculture for farmers community.

Keywords: Application rates; Cotton genotypes; Nutritional qualities; Plant growth; Zinc uptake

Introduction

Cotton, (*Gossypium hirsutum* L.),is an important cash crop and also called as "white gold" of Pakistan as it is ranked among the top five cotton producing countries **[1]**. Although, the overall world fiber production in 1,000 metric tons by country in 2016-2017 **[2]**. The area under cotton crop is greater than before considerably in the last 30years, approximately 7.86 million acres in 2016 **[3]**. Cotton is mostly cultivated in areas with high temperature and humidity, where pest hazards are high especially those pests which reduce the production and its quality. Different strategies of cotton production i.e. High inputs (fertilizer, pest control chemicals, highly drained soil and water) but its utilization affects the environment in various aspects [4]. Soil fertility is the big issue in soils of Pakistan for low crop production, because the lack of precipitation and high evapotranspiration. In Pakistan, freshwater bodies are being polluted through runoff and leaching of nitrates from agricultural land [5] and also overuse and exploitation of chemical pesticides have also harmful impacts on crops and animals as well [6]. To get the maximum yield, computerization has also intensified the use of non-renewable energy. Farm managing practices, agro-ecosystem and soil physicochemical characteristics significantly influence the importance of environmental hazards and resource use in different forms and their effect varies with these practices [7]. In addition, intensive input use, as a form of insurance for cotton production and quality assurance, comes with high production costs. Both environmental hazards and high expenses of cotton production challenge its sustainability and farmer's income in Pakistan; hence, analyzing and quantifying jointly environmental impacts and economic performances of cotton production are compulsory. However, Zinc is an important essential trace element (TE) which performed a key role in enzymatic system of plant [8]. Studied that Zn is important in plant metabolism functions and its deficiency affect plant growth; as a result Zn deficiency has become nutritional constraint for production of crops in various types of soil throughout the world particularly in cereal/grain crops grown on calcareous soil [9]. Zinc deficiency in calcareous is widespread micronutrient disorder in Pakistan, after Nitrogen and Phosphorous Zinc is the third most common deficient nutrient in the country [10]. Every crop needs to be fertilized with balanced amounts of macro and micronutrients to yield better and withstand the stresses. Micronutrients play numerous complex roles in plant nutrition and production. Zinc (Zn) is one of the eight essential micronutrients required for the normal healthy growth and reproduction of crop plants. It functions in many plant enzyme systems as a bridge to connect the enzyme with the substrate upon which it is meant to act [11]. The soils of Pakistan are deficient in Zn approximately 70% especially for rice track areas. Zn dependent enzymes play important roles in structure of proteins and influence hormone release. Being the part of many antioxidant enzymes, Zn helps tolerate environmental and salt stresses. Zn is also necessary for root cell membrane integrity [12]. Studied thatas the root cell membrane permeability is increased under Zn deficiency [13, 11] and external Zn concentrations help mitigate the translocation of Na^+ and Cl^- ions [14]. There are limited research has been done on these studied cotton genotypes, whether the impact of different concentrations of Zinc sulfate may be effective on plant dry matter yield and phyto availability. Hence, the objectives of this study wereto investigate (1) the internal and external Zn-requirement of cotton genotypes and (2) to explore the efficacy of different rates of zinc on the yield and nutritional qualities of cotton genotypes.

Materials and methods

Soil collection

Soil samples were collected from top layer (0-15 cm) of an experimental field of Agronomy research area, Directorate of Agriculture Research, Jaffar abad at Usta Mohammad. The soil was air-dried at the room temperature for 5d and ground to pass through 2 mm sieve and analyzed for their basic physicochemical properties according to [15]. Consequently, sieved samples were stored at 4 °C prior to use and analyzed physicochemical properties of studied soil represented in (Table 1).

Table 1. Basic physicochemical properties of the studied soil

Parameters	Unit	Values
Sand	%	9.5
Silt	%	20
Clay	%	70.5
Textural class		Silty clay
EC	dS m^{-1}	0.89
pH		8.1
CEC	cmol. Kg^{-1}	14
OM	%	1.46
$CaCO_3$	%	13.5

Amendment collection

In this study Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) as soil amendment which was obtained from local market of Usta Mohammad, Baluchistan, Pakistan.

Experimental set-up

A pot experiment was conducted in the wire house, at Directorate of Agriculture Research, Jaffar abad at Usta Mohammad. The experiment was designed to study comparative zinc requirement of cotton (*Gossypium hirsutum* L.) genotypes. Plastic pots were taken and filled with basic experimental soil 5.0 kg of the air dried prepared soil was transferred to each of 36 polyethylene lined plastic pots. This design comprises in a total number of 36 pots (1 soil x 6 treatments x 3 replicates 2 crop).The pots were soaked with approximately 1L applying simple tape water to reach the moisture level about 60 % field capacity (FC).Seeds of cotton (*Gossypium hirsutum* L.) cultivars; NIAB-78 and Bt-802 were sown after delinting. Five seeds were sown in each pot. Thirty six pots were sown with NIAB-78 and second set with Bt-802, half of which were kept controlled while the other half under sodic irrigation. Each of both sets was arranged in six rows with three replications. 1st, 2nd, 3rd, 4th, 5th and 6th rows containing 0, 0.01, 0.02, 0.04, 0.08, and 0.16 mg L^{-1} in soil solution derived from $ZnSO_4.7H_2O$. The plants were arranged in the wire house as a completely randomized design, with the position of plants in the wire house rotated on a weekly basis. The basal dose at 170 kg N, 67 kg P and 67 kg K ha^{-1} were uniformly applied to all pots using urea, DAP and K_2SO_4. Different concentrations of hydrated $ZnSO_4.7H_2O$ were applied viz. Ck, 0.01, 0.02, 0.04, 0.08, and 0.16 mg L^{-1} in soil solution form. The plants were harvested after 50 days of sowing. The shoot samples were removed from each pot and also collected in separate paper bags after washing with distill water and drying with blotting paper.

Soil analyses

Soil texture was analyzed by using the Master sizer 2000E (Malvern, UK) laser diffractometer [16]. The soil EC and pH values were detected in the suspension of (1:2.5 H_2O) by using (DDS-307 EC meter) and (Mettler Toledo 320-S pH meter) followed by (ASTM D1125 and USEPA Method 9045D), respectively. Soil organic matter (OM) was measured by using modified Walkley-Black titration method (ISO 14235, 1998). Total nitrogen (TN) was analyzed by Kjeldahl method (USEPA Method 351.2). Total phosphorus (TP) was detected by spectrophotometrically (Shimadzu UV-VIS 1201), and total potassium (TK) was measured by using flame photometer (Model 420). Total calcium carbonate ($CaCO_3$) was measured with acid neutralization protocol **[17]**. The cation exchange capacity (CEC) was measured by USEPA method 9080.

Laboratory study

Construction of zinc adsorption isotherm and model application

Soil was collected from Agronomy Field, Directorate of Agriculture Research, Usta Mohammad district Jaffar abad. Sub-samples of soil weighing 2.5 g, were shaken with 25 ml 0.01 *M* $CaCl_2$ having different Zn concentrations; 0.2, 0.5, 1.0, 2.5, 5.0, 7.5, 10 and 15 mg L^{-1} as $ZnSO_4.7H_2O$ with three replications each, for 24 hours. After having these samples filtered with Whattman No.41, Zn concentrations in final solution were measured by atomic adsorption spectrophotometer (AAS) following **[18]**. According to **[19]** method sorption isotherm was constructed. After equilibrium Zn amount was calculated from difference of Zn added and remaining in the solution. In order to compute Freundlich equation was used for adsorption data of Zn doses against Zn concentration 0, 0.01, 0.02, 0.04, 0.08, 0.16 mg L^{-1} in soil solution.

Modified Freundlich equation

The sorption isotherm was examined using the modified Freundlich model. The Freundlich model is the adsorption model used for soil P in the literature. Russell and Prescott used the model first in 1961. This is practical model in which similarity terms decreases as the quantity of adsorption is increased. The Freundlich model describes adsorption very well on limited range of concentration **[20]**. Freundlich model equation is described for the study as follows.

$$\frac{x}{m} = K_f(EZC)^{\frac{1}{n}}$$

Where,

K_f =proportionality constant in equation expressed in mg P per kg.

1/n= empirical constant expressed in L per kg.

x/m= the plot between log.

EZC= was drown for soil under investigation.

Plant nutrient analysis

Sample preparation

For full recovery of micronutrients Zn and P from plant samples wet digestion method was used **[21]**. In conical flask one g dried plant sample were taken and kept overnight after addition of 20 ml digestion mixture of di-acid (NHO_3 : $HICO_4$ with 2:1 ratio). Sample was digested next day in hot plate at 150^0C till material was clear. The martial was cooled and diluted with 50ml de-ionized water after digestion and filtered through Whatman filter paper No. 42 and stored in air tight plastic bottles.

Determination of zinc

Digested shoot samples were analyzed for Zn on Atomic Absorption Spectrophotometer (*PERKIN ELMER Aanalyst 100*) was calibrated with a series of respective nutrient standard solutions and standard curve was prepared. Actual concentration in the digest was obtained by comparing the value with standard calibration curve.

$$Micronutrient\ (mgperkg) = \frac{ConcentrationfromCalibrationCurve\ (mgperL)}{WeightofSampleDigested\ (g)}(DilutionFactor)$$

Internal zinc requirement

Internal zinc requirement of cotton was determined from regression equation by plotting the relative yield (95%) against the Zn concentration (%) in the plant.

External zinc requirement

External zinc requirement of cotton was determined from regression equation by plotting the relative yield (95%) against the solution Zn level.

Zinc utilization efficiency

In plant tissues, the biomass generated per unit nutrient concentration is the nutrient utilization efficiency. The following formula was used for the calculation of Zn utilization efficiency.

$$ZUE(g^2 ShootDryMatterper\mu gZn) = \frac{ShootDryMatter\ (g)}{ShootZnConcentration\ (mg/kg)}$$

Parameters studied

Effect of Zn application at different rates on cotton cultivar was evaluated on following plant response variables:

Shoot dry biomass (g/pot).

Zn concentrations in plant shoot biomass (mg/kg).

Zn uptake in plant by shoot biomass (mg/kg).

Shoot Zn utilization efficiency (mg/kg).

Concentration of N.P.K in plant shoot biomass (mg/kg).

Statistical analysis

The experiment was laid out in the two factor factorial CRD with two varieties, 6 Zn treatments, with three replications. All the soil and plant data were statistically analyzed by using complete randomized design and treatment means were compared by using least significance test at $p \leq 0.05$ **[22]**. The pearson correlation coefficients between plant dry biomass, Zn concentration in shoot, Zn uptake in shoot, utilization efficiency and NPK in plant shoot were also performed using IBM SPSS Statistics for Windows, Version 21.0. All the graphs were prepared by using Origin Pro software (version 16).

Results and discussion

As shown in (Table 1), the basic physicochemical characteristics of studied soil. The soil was Silty clay alkaline in reaction, non-saline, moderately calcareous, high in organic matter, and moderate in CEC.

Shoot dry biomass

A number of growth parameters determine the general health of plant amongst which Shoot Dry Biomass is the most sensitive response parameter of plant towards nutrient deficiency or toxicity and has a vital role in screening experiments **[23]**. Data about Shoot Dry Biomass (SDB) of two different cotton varieties produced at different levels of Zn under normal condition, as compare to the control the maximum plant dry biomass was observed by 31.64% with Zn-0.04 mg L^{-1}application rate by NIAB-78 and 35.82% with Zn-0.04 mg $L^{-1}$by BT-802, whereas Zn-0.16 mg L^{-1} reduced up to 1.85% by NIAB-78 and 2.32% with Zn-0.16 mg L^{-1} by BT-802 respectively (Figure 1a). Our results are in good agreement with **[24]** who reported that cotton yield significantly increased with addition of boron and Zn alone and in combination. Similarly, **[25]** concluded that application of Zn (0, 5, 10, 15, and 20 mg Zn kg^{-1} soil) evidently promoted maize yield as well as uptake by plant with increasing Zn rates. However, **[26]** conducted two field experiments on rainfed wheat grown on alkaline Zn-deficient *Typic Haplustalfs* (AB-DTPA; Zn, 0.49 to 0.52 mg kg^{-1} soil). Soil applied Zn increased grain yield up to 12% over control.

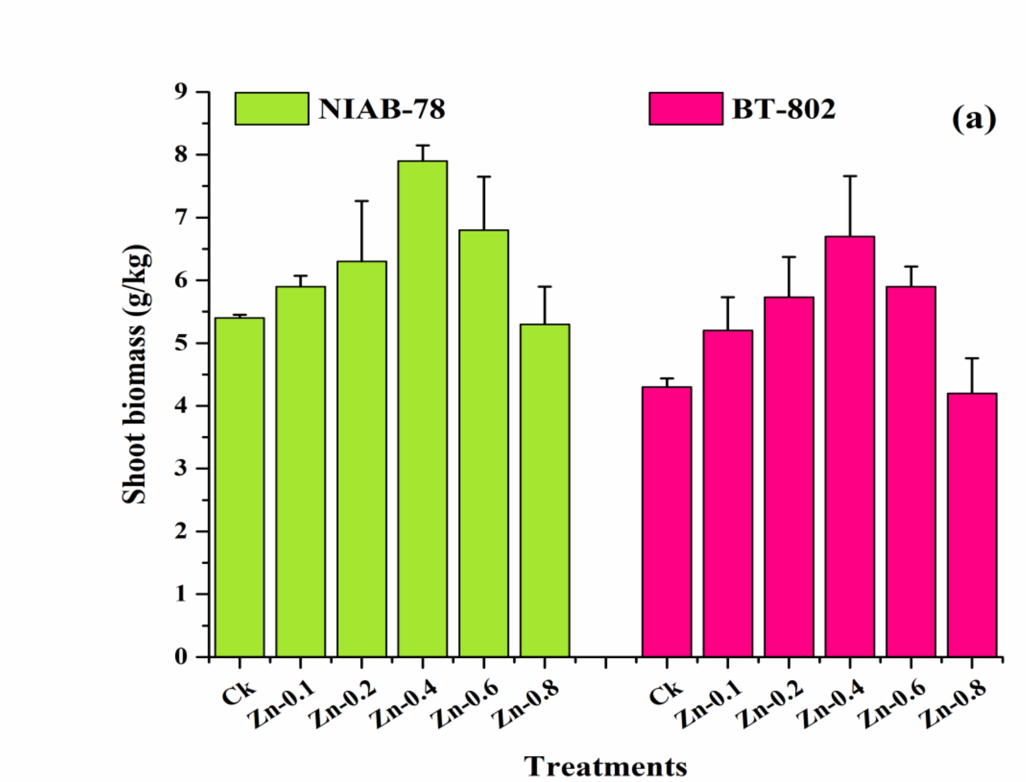

Figure 1a.Shoot dry matter (g/pot) of cotton cultivars at different Zn levels

Shoot Zn concentration (mg/kg)

Zinc (Zn) is one of the eight essential micronutrients required for the normal healthy growth and reproduction of crop plants. It functions in many plant enzyme systems as a bridge to connect the enzyme with the substrate upon which it is meant to act **[11]**. Application of amendment gradually increased the Zn concentration in cotton genotypes as compared to control. However, the maximum accumulation of Zn in plant shoot biomass was observed by 83.69 with Zn-0.16 mg L^{-1} in NIAB-78 and 86.89% with Zn-0.16 mg $L^{-1}$by BT-802 respectively (Figure 1b). Similarly, Zn is also necessary for root cell membrane integrity **[12]** as the root cell membrane permeability is increased under Zn deficiency **[13, 11]** and external Zn concentrations help mitigate the translocation of Na^+ and Cl^- ions **[14]**. However, cotton cultivars vary in their ability to accumulate Zn in their body **[27]**. This result is in harmony with the previous studies in wheat in which seed Zn absorption was enhanced by foliar Zn addition about three folds as compared to control **[28]**.

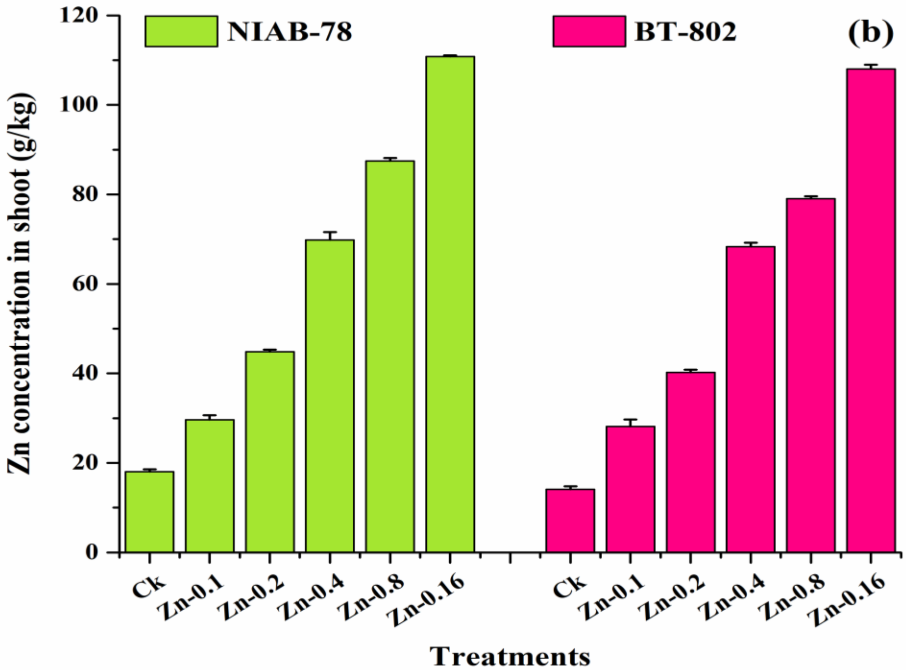

Figure 1b. Shoot zinc concentration (mg/kg) in cotton cultivars at different Zn levels

Zinc uptake in shoot biomass(mg/kg)

Impact of the soil amendment at different concentration significantly (*P*<0.05) enhanced the Zn uptake in plant shoot biomass by cotton genotype as compared to un-amended treatment. Indeed the maximum Zn uptake in plant shoot was received by 88.96% with Zn-0.16 mg L^{-1} in NIAB-78 and 90.28% with Zn-0.16 mg L^{-1} by BT-802 respectively (Figure 1c). Similarly, **[24, 27, 29]**, also observed an increase in Zn uptake by plants with increasing Zn application rate.

Zinc utilization efficiency (mg/kg)

Zinc levels had significant (p<0.01) effect on Zn utilization efficiency (Zn UE) which may be helpful in identifying plant cultivars that can yield better under low concentration of a particular nutrient **[30, 31]**. This study demonstrated the effectiveness of different Zn levels for improving Zn utilization in cotton. Zinc utilization efficiency was significantly (P<0.05) increased with application soil amendment. Consequently, as compared to control the maximum shoot Zinc utilization efficiency was observed 87.35% with Zn-0.16 mg L^{-1} by NAIB-78, and 88.60% Zn-0.16 mg L^{-1} by BT-802 correspondingly (Figure 1d).

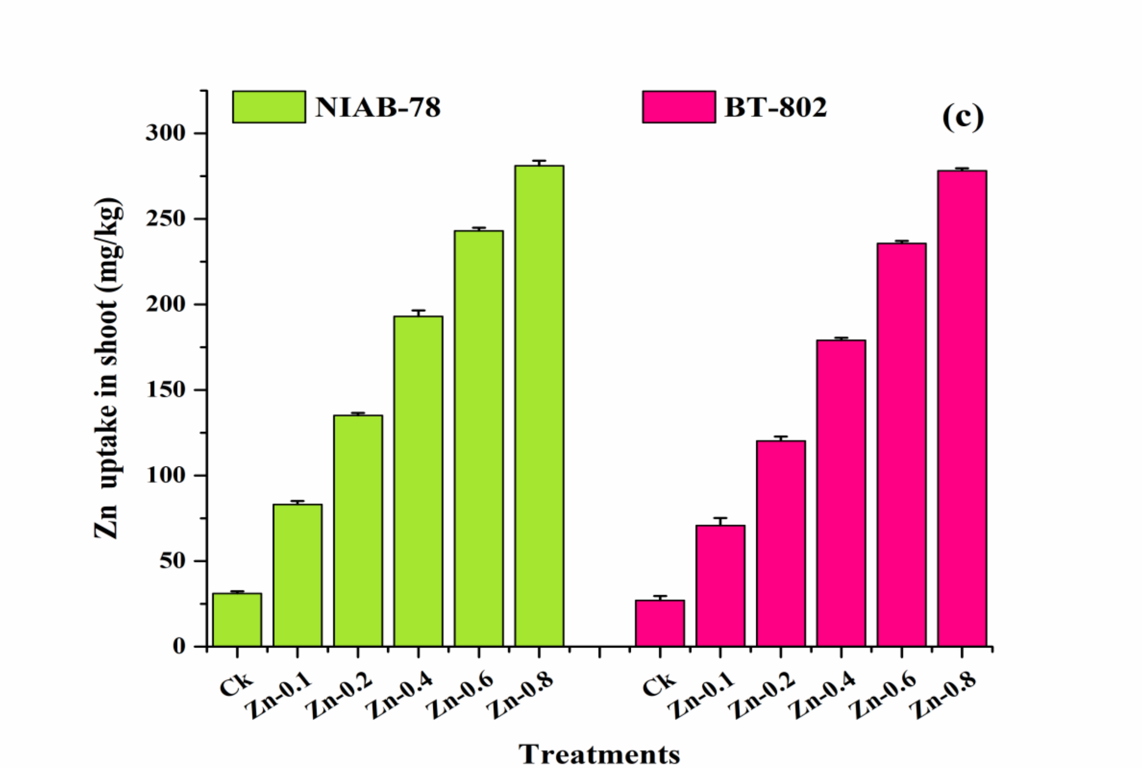

Figure 1c. Shoot zinc uptake (mg/kg) in cotton cultivars at different Zn levels

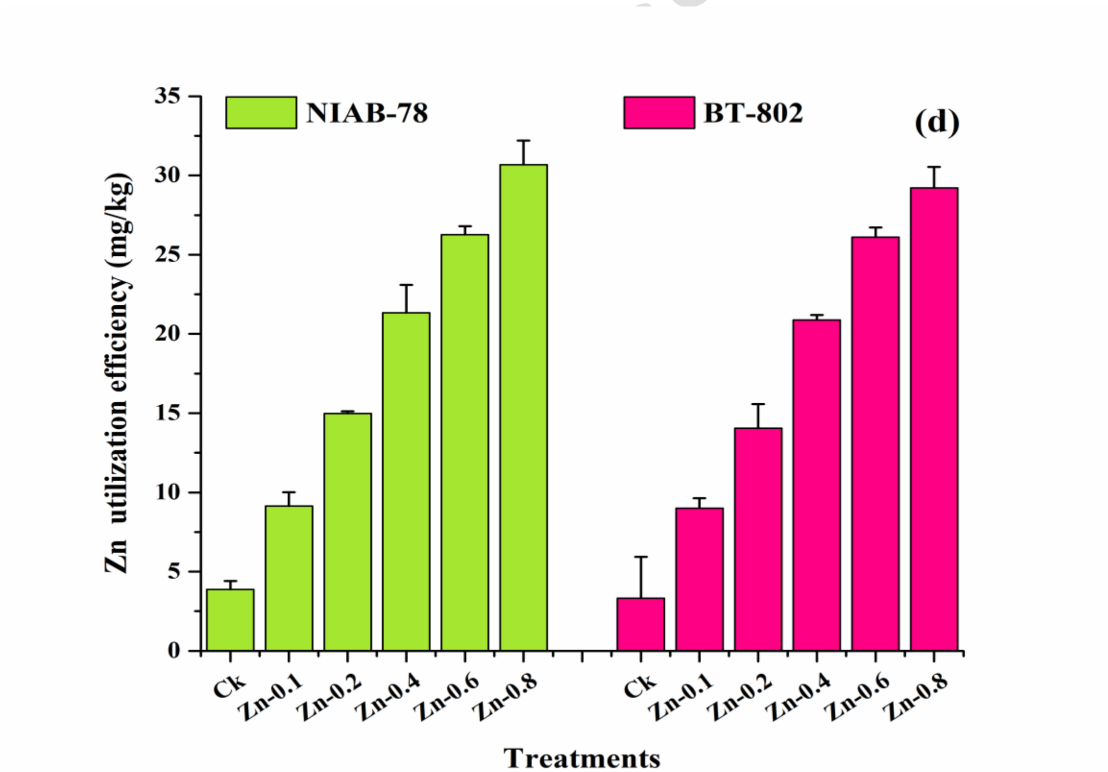

Figure 1d. Zinc utilization efficiency (mg/kg)in cotton cultivars at different Zn levels

Concentration of NPK (mg/kg)
NPK concentration in shoot (mg/kg) of two cotton cultivars is presented in (Figure 1e) the maximum NPK concentration was observed in NIAB-78, whereas minimum was recorded in Bt-802. Similarly [32] reported that with addition of zinc sulfate promoted nutrient (NPK) uptake, utilization, and metabolism, somewhat improved root and shoot biomass, bloom, dry matter yield, and enhanced cotton quality.

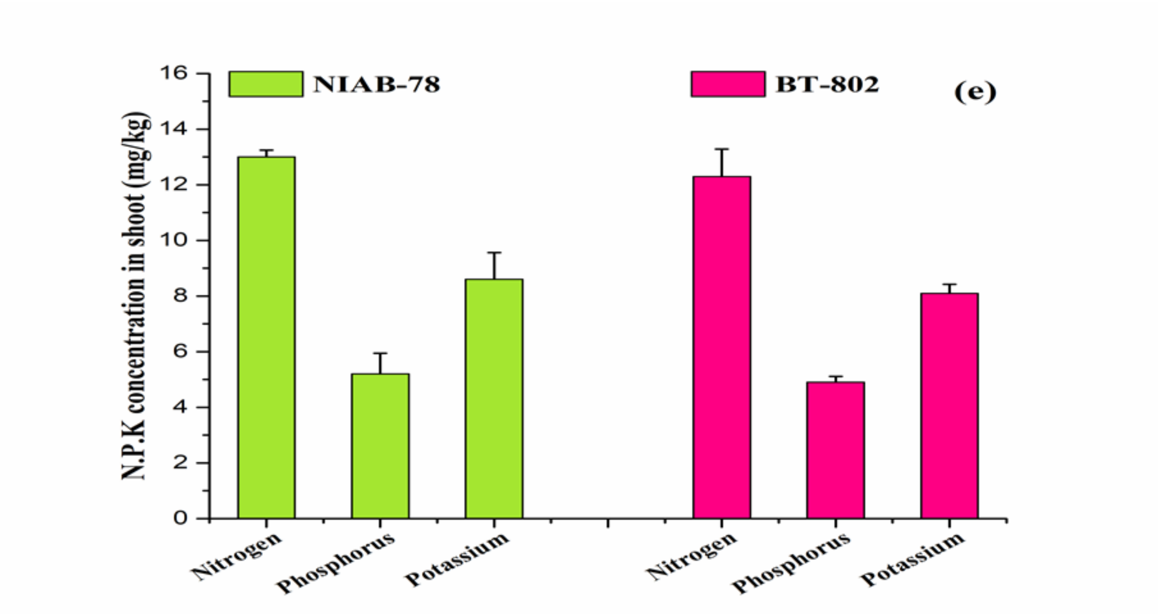

Figure 1e.Shoot concentration of NPK (mg/kg) in cotton cultivars

Pearson correlation analysis
Pearson correlation analysis was performed to assess the relationship between different studied traits (Table 2). NIAB-78 shoot biomass was highly correlated with BT-802 shoot biomass. Furthermore, Zn concentrations, uptake in NIAB-78 and/or BT-802 shoot biomass and Zn utilization efficiency in NIAB-78 shoot were highly correlated with Zn concentration in BT-802 shoot, Zn uptake in NIAB-78 and/or BT-802 shoot, Zn utilization efficiency in NIAB-78 and/or, BT-802 shoot biomass. Moreover, NPK in NIAB-78 shoot was also positively correlated with NPK in BT-802 shoot biomass. Similar to our results [33] reported that the benefit of high seed Zn in seedling growth is also highlighted by a positive correlation among Zn content in germinating seeds and the collective root and shoot dry matter yield (r = 0.55, p< 0.05). Zinc in rice grains can be efficiently increased by foliar Zn addition after flowering, with a possible advantage of this to rice eaters indicated by approximately 55% increases of brown rice Zn, and agro-nominally in more rapid early growth and establishment.

Table 2. Pearson correlation coefficients between on plant dry biomass, Zn concentration in shoot, Zn uptake in shoot, utilization efficiency and NPK in plant shoot

Parameters	NIAB-78 shoot biomass	BT-802 shoot biomass	Zn concentration in NIAB-78 shoot	Zn concentration in BT-802 shoot	Zn uptake in NIAB-78 shoot	Zn uptake in BT-802 shoot	NIAB-78 Zn utilization efficiency	BT-802 Zn utilization efficiency	NPK in NIAB-78 shoot	NPK in BT-802shoot
NIAB-78 shoot biomass	1									
BT-802 shoot biomass	.967**	1								
Zn concentration in NIAB-78 shoot	.164	.074	1							
Zn concentration in BT-802 shoot	.164	.068	.997**	1						
Zn uptake in NIAB-78 shoot	.257	.191	.990**	.985**	1					
Zn uptake in BT-802 shoot	.220	.147	.995**	.989**	.999**	1				
NIAB-78 Zn utilization efficiency	.259	.190	.991**	.986**	1.000**	.998**	1			
BT-802 Zn utilization efficiency	.286	.217	.987**	.981**	.999**	.997**	.999**	1		
NPK in NIAB-78 shoot	-.614	-.679	-.498	-.599	-.563	-.532	-.538	-.591	1	
NPK in BT-802 shoot	-.617	-.681	-.501	-.602	-.566	-.536	-.541	-.594	1.000**	1

Note: Correlation is significant at the **p*<0.05; ***p*<0.01 level. Table 2 indicated the different concentration of Zinc sulfate on plant dry biomass, Zn concentration in shoot, Zn uptake in shoot, utilization efficiency and NPK in plant shoot

Conclusions and recommendations

The results obtained revealed that Zn-0.16 mg L^{-1} in soil solution was highly effective in promoting plant dry matter yield. Furthermore, Zn concentration in plant shoot, uptake in plant leaf tissue and utilization efficiency was increased the addition of amendment dose. Overall, the total content of NPK in plant shoot biomass in NIAB-78 was higher than the BT-802 genotype. On the basis of these results this study should be confirmed in long term field condition.

Authors' contributions

Conceived and designed the experiments: A Mastoi, Performed the experiments: ZA Mastoi & AS Khetran, Analyzed the data: AS Khetran & Rahmatullah, Contributed reagents/ materials/ analysis tools: JA

Hassni & M Rasheed, Wrote the paper: GA Baloch.

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