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Response of Rice (*Oryza sativa* L.) to Soil and Foliar Application of Nano-ZnO and Bulk Zn-fertilizer in Red Acidic Soil of West Bengal, India

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IN CEREAL crops Zinc (Zn) deficiency has become a major problem which causes reduction in yield and nutritional quality of the cereal grain, thus affecting human health. Being an essential micronutrient for the production of rice (*Oryza sativa* L.) crop, Zn deficiency appears to be an acute problem for the human population whose staple food is rice. Small increase in the nutritive value of rice can thus contribute to the human nutrition. Thus, increasing the micronutrient content of a food crop with the help of enriched fertilizers appears to be an effective way. During two successive growing seasons (wet season, 2017 and dry season, 2018) at the experimental farm of PalliSiksha Bhavana, Visva-Bharati, West Bengal, India, field experiments were carried out to assess the potential of Zn fertilizer on plant height, grain quality, leaf chlorophyll and leaf Zn content of rice plant variety MTU-1010 by application of foliar Nano-ZnO (Zinc oxide) and soil $ZnSO_4.7H_2O$ (Zinc Sulphate Hepta Hydrate). In both seasons, each experiment was conducted in a randomized block design (RBD) with three replicates. Soil Zn as $ZnSO_4.H_2O$ applications were assigned in the plots at the rate of 5 kg/ha and 0.30% nano-ZnO were assigned as foliar Zn spray along with the basal doses of 80 kg nitrogen (N), 40 kg phosphorus (P_2O_5) and 40 kg potassium (K_2O)/ha as per the treatment details. After 15 days, 30 days, 45 days from transplanting and at the time of flowering as well as post-flowering chlorophyll (a, b, a+b) and Zn in rice plant leaves were determined. Plant heights were recorded after 30 days, 60 days and 90 days from transplanting and at the time of harvest. Quality parameters such as amylose, starch and crude protein content of the grain samples were determined at harvest stage. The results obtained from present assessment showed that, application of soil as well as foliar Zn resulted in significant increase in leaf Zn content, chlorophyll content, grain crude protein and plant height compared to the control. No significant effect on starch and amylose was recorded with Zn fertilization. Foliar spraying with 0.30% nano-ZnO at the time of flowering and post flowering showed a significant augmentation in leaf Zn concentration, chlorophyll content, plant height and quality of rice grain compared to the treatments receiving soil Zn application. Therefore, it is considered as a more beneficial treatment in the cultivation of rice plants to promote plant growth and quality.

Keywords: Zn fertilization, *Kharif* and *boro* rice, Leaf chlorophyll and Zn content, Plant height, Quality of rice.

Introduction

Zn deficiency has been addressed as a public health problem in the developing world, giving rise to severe health complications and socio-economic problems (Hotz & Brown, 2004; Stein et al., 2007). Series of metabolic disorders in human body are seen in Zn deficiency condition which eventually can lead to death (Mayer et al., 2008; Wang et al., 2021). More than 3 Billion people are currently suffering from Zn deficiency, worldwide (Cakmak et al., 2010). Sadak & Bakry (2020) reported that Zn is an essential micronutrient for the growth and productivity of plants. Many enzymatic reactions

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get activated by Zn (Vitosh et al., 1994; Pedler et al., 2000). Zn plays important role in several plant physiological processes such as carbohydrate metabolism, respiration, gene expression and its regulation (Klug & Rhodes, 1987). These physiological processes get disrupted due to Zn deficiency. Photosynthesis, chlorophyll synthesis, protein quality, nitrogen uptake and nitrogen metabolism are the processes where major role is played by Zn (Alloway, 2008; Cakmak, 2008; Potarzycki & Grzebisz, 2009). Zn is an important component of carbonic anhydrase and stimulator of aldolase which are involved in carbon metabolism (Tsonev & Lidon, 2012; Hassan et al., 2020). Being an integral component of several biomolecules such as proteins, lipids, co-factor of auxins, it plays major role in plant nucleic acid metabolism (Hassan et al., 2020).

The most common cause of Zn deficiency in humans is malnutrition which is due to insufficient intake of bio available Zn from foods (Maret & Sandstead, 2006; Lazarte et al., 2016; Wang et al., 2021). By eating animal based diet Zn deficiency can be addressed but here come the limiting factors such as religious backgrounds and financial factors (Bhatt et al., 2020). Lakshmi et al. (2021) reported that the major concern in human diets is the adequacy in grain Zn in staple food crops like rice and wheat. Zn deficiency appears as a major threat for the human population whose staple food is rice (Stein, 2010; Mandal & Ghosh, 2020). The sustainable strategy to solve the problem of Zn deficiency in humans is to enhance grain Zn concentration in staple food crops (Haider et al., 2021; Khampuang et al., 2021). Researchers (Phattarakul et al., 2012; Wang et al., 2017; Wang et al., 2021) reported various strategies to improve grain Zn bioavailability by increasing grain Zn concentration. Grain nutrient content can be enriched following agronomic biofortification (Cakmak, 2008) which is preferred over genetic biofortification as the former process is a short term process (Pfeiffer & McClafferty, 2007; Cakmak et al., 2010). Thus, Zn biofortification of rice appears as a viable option to combat Zn-deficiency. If biofortification technique is being employed properly and timely, current global challenge of mineral malnutrition can be avoided (Das et al., 2019, 2020; Hossain et al., 2019; Bhatt et al., 2020). For improving Zn uptake by rice crop, grain yield, nutritional quality of grain, the most common agronomic practice is to apply Zn fertilizer to the soil or on foliage (Li et al., 2015; Khampuang et al., 2021). Foliarly applied Zn appears as an effective solution to achieve increased Zn concentration in grain even on potentially deficient soils (Bhatt et al., 2020), thus providing nutritional benefits to consumers. The production of biofortified crops enriched with micronutrient Zn has been reported by several researchers (El-Ramady et al., 2021; Silva et al., 2021).

Amongst various Zn fertilizers available, Zn sulphate hepta hydrate ($ZnSO_4.7H_2O$) containing 21-22% of Zn, 11% of S and 43% of H_2O is found to be most efficient, readily available, economically cheapest for correcting Zn deficiency in most of the crops and diverse soils compared to sparingly soluble Zn sources, chelates and mixtures among various inorganic sources. Apart from the bulk fertilizers, the invension of nano fertilizers have gathered attention in recent times. The term nano means 10^{-9} of one billionth of something (Abd-Elrahman & Mostafa, 2015). Nano materials are composed of small size materials and these components show properties at the micro level (Abd-Elrahman & Mostafa, 2015). Nano fertilizers have unique physicochemical properties and the potential to boost the plant metabolism (Giraldo et al., 2014). The nano fertilizers or nano encapsulated nutrients might have the properties that are effective to crops, release the nutrients on demand, controlled release of chemical fertilizers that regulate the plant growth and enhanced target activity (DeRosa et al., 2010). The technique nano-biofortification can be employed by applying nano-ZnO for correcting Zn deficiency in crops. In this technique, metal or metal oxide nanoparticles can be used for the production of biofortified crop (El-Ramady et al., 2021; Velazquez-Gamboa et al., 2021). Amongst the various strategies adopted across the world for alleviating Zn deficiency in crops, nanofertilizers show better nutrient use efficiency due to its unique characteristics over bulk fertilizers.

Rice is considered one of the most important food crop in the world providing staple food to the millions (Hussien et al., 2020; Singh et al., 2020). In respect of area and production of rice, India ranks second following China (FAO, 2013). In India, rice is the first most important crop, it is being grown in an area of 43.79 million/ha with a total production of 112.91 million tones and an average production of 2578 kg/ha (Anonymous, 2018; Singh et al., 2020). India comes first in terms of area (44.5 million ha) and second in production (172.58 million tonnes) (FAO, 2018).

To describe the health of agricultural soils, the term soil quality has been introduced which

is considered as an indicator for evaluating sustainability of soil and crop management practices (Gregorich et al., 1994; Doran & Zeiss, 2000; El-Halim, 2013). Soil testing provides the information about the nutrient status of the soil (Mandal & Ghosh, 2021). Researchers reported that paddy soil conditions are not adequate for attainable Zn usually, hence Zn insufficiency has been noted in rice soils (El-Hissewy et al., 2016; Mosaad, 2019). World wide it has been found that 30% of the soils are poor in plant available Zn (Alloway, 2008; Mosaad, 2019). In West Bengal, red and lateritic soils are observed in the districts Birbhum, Bankura, Burdwan, Midnapore and Purulia which occupy about 28,000 sq km. 28% of the geographical area of the state is occupied by these districts (Anonymous, 1989). Panda et al. (1991) reported that red, laterite and associated soils of Eastern India are characterized by low organic matter, low phosphorus (P) content, acidic soil pH, light textured and are often deficient in available Zn. When rice is being cultivated in Zn deficient soil, poor yield is obtained coupled with poor quality crops. The major reasons for Zn deficiency in soils can be attributed to the imbalanced fertilizer application, low soil organic matter, high yielding crop varieties in past (Pal et al., 2020; Kumar & Ram, 2021). Manojlović et al. (2019) reported that intensive uses of minerals by crops have resulted in rapid depletion of micronutrient reserves including Zn from soil causing micronutrient deficiency.

Therefore, the aim of the present investigation was to study the response of rice to soil and foliarly applied nano-ZnO and bulk Zn-fertilizers in red acidic soil of West Bengal, India.

Materials and Methods

Field experiments

Field experiments were conducted at Experimental farm, PalliSiksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, West Bengal, India (87°42' E, 23°39' N, 58.9 m above mean sea level) during dry season (*kharif*) of 2017 and wet season (*boro*) of 2018 on rice (*Oryza sativa* L.). Eight treatments were arranged in a completely randomized block design with three replications. Description of treatments are given, T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering. Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. Soil and foliar Zn were applied through $ZnSO_4.7H_2O$ and nano-ZnO respectively, as per the treatment details.

Crop studies

Data on plant height (cm) were recorded at 30 DAT, 60 DAT, 90 DAT and at harvest. Chl. a, Chl. b and total chlorophyll (mg/g) contents were determined as per the procedure suggested by Hiscox & Israelstam (1979). At full maturity, crops were harvested and air dried. Grains were cleaned and sun dried until constant weight was recorded.

Plant chemical studies (Rice Grain samples)

Plant leaf samples collected at various growth stages of rice crop were digested using diacid mixture (HNO_3: $HClO_4$:: 9:4; Jackson, 1973) and analyzed for Zn content as per the procedure suggested by Lindsay & Norvell (1978) using Atomic absorption spectrophotometer. Modified Kjeldahl method (Jackson, 1973) was employed for the determination of total N content of plant grain samples. Quality parameters such as amylose, starch and crude protein content of the grain samples were determined. Crude protein content (%) of plant grain samples were determined by multiplying grain N content by a factor 6.25 (AOAC, 2000). Grain amylose and starch contents were determined as per the procedure described by Sadasivam & Manickam (1992) and expressed in percentage.

Soil sample analysis

Composite soil samples were collected at 0-15 cm depth before start of the experiment, air dried, powdered and were analyzed for physico-chemical properties. Particle size analysis and textural class was determined using Hydrometer method (Bouyoucos, 1927). Soil pH and electrical conductivity were determined following the method as suggested by Jackson (1973). Wet digestion method was employed for soil organic carbon content analysis (Walkley & Black, 1934). Available N content in soil was estimated using Kjeldahl distillation process (Subbiah & Asija, 1956). Bray's No. 1 method was followed for determining available P (Bray & Kurtz, 1945). Ammonium acetate (CH_3COONH_4) was used for extracting available potassium (K) (Jackson, 1973) and readings were taken in flame photometer. 0.005 M DTPA solution (0.005 M Diethylene Triamine Penta Acetic acid + 0.1 M Triethanol amine +

0.01 M $CaCl_2$), adjusted to pH 7.3 ± 0.05 was used as the extractant (1:2 soil to extractant ratio) to extract plant available Zn content (Lindsay & Norvell, 1978) with the help of Atomic absorption Spectrophotometer (AAS). Experimental soil was silt loam in texture with pH 5.16, EC 0.09 dS/m, organic carbon 0.38% (low according to Muhr et al., 1965), low in available N 175.62 kg/ha, medium in available P 13.40 kg/ha, low in available K 85.53 kg/ha (according to the rating chart of the macronutrients N, P and K as given by Arora, 2002) and sufficient in DTPA-extractable Zn 1.22 mg/kg (according to Lindsay & Norvell, 1978).

Statistical analysis

Statistical analysis of the data was carried out following Analysis of Variance (ANOVA) technique using statistical software IBM SPSS25 and Microsoft office Excel 2007 at 5% probability level to determine the difference among treatment means.

Results and Discussion

The results regarding the effect of Zn application (soil application of $ZnSO_4.7H_2O$ as well as foliar application of nano ZnO) on growth, chlorophyll content and leaf Zn content of rice are presented as under. Data showed that foliar application of ZnO and soil application of $ZnSO_4.7H_2O$ positively improved growth, chlorophyll content, leaf Zn content of rice grown in lateritic soil.

Plant height at 30, 60, and 90 DAT and harvest of rice

Plant height is considered to be one of the most important growth and development indicators. Plant height is an important morphological attribute; it is a function of combined effects of genetic make up of a plant, soil nutrient status, seedling vigor and the environmental conditions under which it is grown (Mustafa et al., 2011). Blair & Lefroy (1987) reported that Zn application significantly affected plant height at all growth stages but magnitude was not same, it increased with advancement of growth up to anthesis and then it got slowed down. The data related to plant height recorded at 30, 60, and 90 days after transplanting and at harvest as influenced by Zn application at various growth stages of rice crop during 2017, 2018 and pooled of two years is presented in Table 1. Zn application at various growth stages significantly increased plant height ($P < 0.05$) in comparison to control (T_1) (Fig. 1). At four successive growth stages, plant height measurements were done. The plant height of MTU-1010 rice was significantly affected by the application of Zn at different growth stages. In the present study Zn sulphate hepta hydrate ($ZnSO_4.7H_2O$) was applied through soil and foliar application of nano Zn oxide (ZnO) was done. All the Zn applied treatments showed significantly taller plants over no Zn application (T_1) at all intervals of crop growth. The enhanced plant height with Zn application might be due to the adequate supply of Zn which helps accelerating enzymatic activity and auxin metabolism in plants (Maqsood et al., 1999).

TABLE 1. Plant Height (cm) at 30 DAT, 60 DAT, 90 DAT and harvest as influenced by Zn application at various growth stages of kharif and boro rice

Treat.	Plant height (cm) 30 DAT			Plant height (cm) 60 DAT			Plant height (cm) 90 DAT			Plant height (cm) harvest		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	55.35d	53.67e	54.51d	76.52b	75.68b	76.10b	86.51b	83.53b	85.02b	95.35b	94.90b	95.13b
T_2	61.13bc	61.89ab	61.51b	85.64a	82.54a	84.09a	93.02a	92.94a	92.98a	102.85a	104.10a	103.47a
T_3	65.32a	60.61bc	62.96ab	85.94a	82.89a	84.42a	91.90a	91.31a	91.60a	103.28a	103.79a	103.53a
T_4	60.92c	59.04cd	59.98c	85.86a	82.63a	84.24a	92.31a	92.34a	92.32a	103.52a	103.23a	103.38a
T_5	62.65b	63.82a	63.23a	86.42a	83.03a	84.72a	92.14a	92.88a	92.51a	104.07a	104.14a	104.10a
T_6	61.22bc	61.98ab	61.60b	86.05a	80.89a	83.47a	90.97a	91.71a	91.34a	103.44a	104.72a	104.08a
T_7	60.72c	62.82ab	61.77b	86.50a	81.47a	83.99a	92.72a	92.80a	92.76a	102.04a	101.56a	101.80a
T_8	59.72c	58.07d	58.90c	84.44a	81.26a	82.85a	93.23a	92.53a	92.88a	101.91a	103.53a	102.72a

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

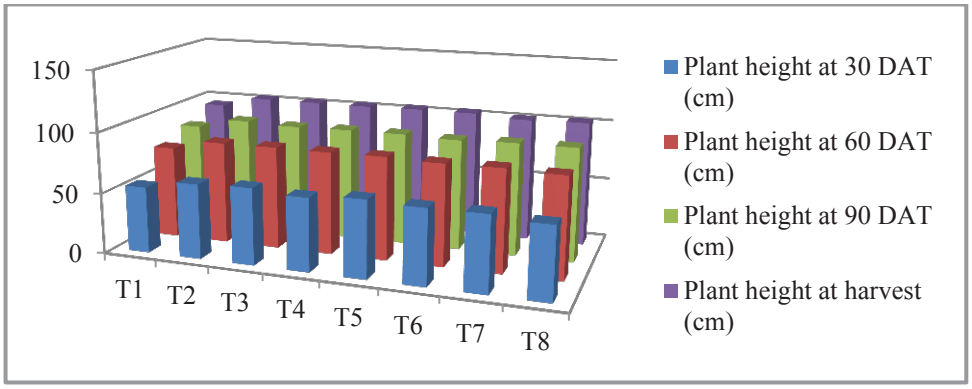

Fig. 1. Plant Height (cm) at 30 DAT, 60 DAT, 90 DAT and harvest as influenced by Zn application at various growth stages of kharif and boro rice [Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering. Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26]

Pooled data showed that at 30 DAT plant height ranged from 54.51 to 63.23 cm, while it ranged from 76.10 to 84.72 cm at 60 DAT, 86.02 to 92.98 cm at 90 DAT and from 95.13 to 104.10 cm at the time of harvest. The heights of plants among different treatments vary significantly at 30 DAT, 60 DAT, 90 DAT and at the time of harvest as well. At 30 DAT, highest plant height (63.23 cm) was recorded in treatment T_5 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ after 25 days of transplanting) which was statistically *at par* with treatment T_3 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ after 25 days of transplanting) with value of 62.96 cm. Lowest plant height (54.51 cm) was recorded in plots treated with no Zn (T_1). All the Zn applied treatments resulted significantly higher plant height in comparison to control.

At 60 DAT, highest plant height was recorded in treatment T_5 (84.72 cm) (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ after 25 days of transplanting) which was statistically *at par* with treatments T_2 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of land preparation), T_3 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ after 25 days of transplanting), T_4 (NPK + Zn @ 5 kg Zn/ha (Flowering) as $ZnSO_4.7H_2O$ at the time of flowering), T_6 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ at the time of flowering) and T_7 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half after 25 days of Transplanting + half $ZnSO_4.7H_2O$ at the time of flowering) with values of 84.09 cm, 84.42 cm, 84.24 cm, 83.47 cm and 83.99 cm respectively. Lowest plant height (76.10 cm) was recorded in control (T_1).

At 90 DAT highest plant height was recorded in treatment T_2 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of land preparation) (92.98 cm) and lowest plant height (85.02 cm) was recorded in control T_1. The effect of Zn application at various growth stages of rice on plant height at 90 DAT was significant ($P < 0.05$). All the Zn applied treatments produced significantly higher plant height in comparison to control. Treatment T_2 remained statistically *at par* with treatments T_3, T_4, T_5, T_6, T_7 and T_8 (NPK + 0.30% nano Zn oxide spray at the time of flowering and post-flowering) with values 91.60, 92.32, 92.51, 91.34, 92.76 and 92.88 cm, respectively.

At harvest, the highest plant height value (104.10 cm) was received for the treatment T_5 while the lowest plant height (95.13 cm) was recorded in control treatment (T_1). T_5 was found statistically *at par* with treatments T_2, T_3, T_4, T_6, T_7 and T_8 with values of 103.47 cm, 103.53 cm, 103.38 cm, 104.08 cm, 101.80 cm and 102.72 cm, respectively. 9.43% increase of plant height was recorded in treatment receiving split application of 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of land preparation and 25 days after transplanting (T_5) compared to control at the time of harvest. Thus, it can be concluded from the above results that the effect of different time of foliar as well as soil Zn application on plant height was significant. Plant heights were

increased significantly with foliar application of nanofertilizers. The results indicated that Zn fertilization had significant effect on plant height.

Increase in the plant height with Zn dose might be due to the auxin metabolism as reported by Srinivasan & Naidu (1986). They further reported in their studies that Zn influences the shoot length most probably through auxin metabolism. Active vegetative step in the rice continues up to tillering step, thus vegetative growth in the said step increases with the more speed (Srivastava et al., 1999). Hence foliar application at tillering step has more profound effect on plant height compared to that of initiation step of panicle formation. Zn fertilization increases plant height significantly by increasing distances of internodes (Kaya et al., 2000). Alam & Shereen (2002) observed that there was increase in shoot length in wheat in all treatments in comparison to control while studying the effect of different levels of Zn and P on wheat during water culture experiment. Foliar application of Zn, can improve the growth of the rice through increasing the concentration and its transfer through plant sap results in increasing growth and development of rice (Hacisalihoglu & Kochian, 2003). Zn deficiency prevents auxin synthesis and this causes reduction in plant height (Zand et al., 2007). Chaudhary et al. (2007) reported that Zn plays important role in the growth and development of rice. Khan et al. (2007b) reported that rice plant height was affected significantly with Zn application. Highest plant height (111.3 cm) was recorded with Zn application at the rate of 5 kg/ ha. Khan et al. (2007a) reported that all methods of Zn application resulted significant increase in plant height which might be due to increased enzymatic activity and auxin metabolism in plant. Similar results were given by Abdoli et al. (2014). Significant effect of Zn application on increasing rice plant height has been reported by Hajiboland & Beiramzadeh (2008), Jiang et al. (2008), Sarwar (2011). Due to the involvement of Zn in different physiological processes such as chlorophyll formation (Habib, 2009), enzyme activation (Yassen et al., 2010), stomatal regulation (Oosterhuits & Weir, 2010), increase in plant height was observed. Movahhedy-Dehnavy et al. (2009) reported that IAA synthesis increases with Zn application which increase plant height by increasing internode length and number of panicle. Plant height and other growth parameters of rice plant was significantly increased with Zn fertilization over plots treated with no Zn (Shivay et al., 2010). Plant height response to Zn application had more profound effect, higher plant height was recorded with Zn application and lowest was recorded without Zn application (Sarwar, 2011). Highest plant height (101.70 cm) at maturity was recorded with application of 6 kgZn/ha, whereas corresponding lowest plant height (78.5 cm) was recorded with no application of Zn (Muthukumararaja & Sriramachandrasekharan, 2012). Application of Zn at 5 kg/ha recorded significantly higher plant height over no application of Zn (Singh et al., 2012). Researchers reported that different micronutrients as nanoparticles had affected plant growth (Bala et al., 2014; Valadkhan et al., 2015). Alam & Kumar (2015) reported that plant height at 60 DAT was increased with the application of Zn at the rate of 5 kg/ha (77.20 cm in the first year; 79.88 cm in the second year) in comparison to control (58.54 cm in the first year; 60.64 cm in the second year). At 80 DAT also Zn application at the rate of 5 kg/ha (94.56 cm in the first year; 97.95 cm in the second year) increased plant height in comparison to control (86.76 cm in the first year; 89.80 cm in the second year). Mahmoodi & Mogadam (2015) reported that applying foliar Zn at tillering step has more profound effect on rice plant height compared with initiation step of panicle formation. Ghoneim (2016) reported that Zn application affected plant height significantly. Similar result was given by Jatav & Singh (2018). Sharma et al. (2016) reported that significant increase in plant height was recorded in Zn treated plots in comparison to control. As Zn plays important role in growth and development of plants because of its catalytic and stimulatory effect in various metabolic and physiological processes of plants, its application causes significant increase in plant height. Singh et al. (2017) reported that Zn application at the rate of 5kg/ha increased plant height recorded at 30DAT, 60DAT, 90DAT and at the time of harvest in comparison to control.

Chlorophyll contentof plant leaf samples

The data regarding chlorophyll a, chlorophyll b and total chlorophyll (mg/g) in plant leaves as influenced by Zn application at various growth stages during 2017, 2018 and pooled of two years is presented in Tables 2 to 6. Pooled data showed that Zn application at various growth stages of rice crop has significantly affected chlorophyll content of rice plant leaves. Fig. 2 indicates that Zn fertilization increased leaf Zn content and chlorophyll content of rice leaf samples proportionally. Chlorophyll a content varied from 0.85-1.10 mg/g at 15 DAT, 0.88-1.14 mg/g at 30 DAT, 0.89-1.15 mg/g at 45 DAT, 0.90-1.25 mg/g at the time of flowering and 0.89-1.27 mg/g at the time of post-flowering. The data further

illustrated that chlorophyll a content increased with the addition of Zn at various growth stages. Chlorophyll b content ranged from 1.45-1.89 mg/g at 15 DAT, 1.53-1.96 mg/g at 30 DAT, 1.50-1.98 mg/g at 45 DAT, 1.53-2.04 mg/g at the time of flowering and 1.50-2.20 mg/g at the time of post-flowering. Application of Zn increased chlorophyll b content at various growth stages. Zn applied treatments showed higher chlorophyll content in comparison to control (T_1) which is having lowest chlorophyll content at all intervals. Total chlorophyll content also increased with soil as well as foliar Zn application. Total chlorophyll content ranged from 2.31-3.00 mg/g at 15 DAT, 2.41-3.10 mg/g at 30 DAT, 2.40-3.12 mg/g at 45 DAT, 2.43-3.29 mg/g at the time of flowering and 2.39-3.48 mg/g at the time of post-flowering.

TABLE 2. Chlorophyll content (mg/g) of plant leaf samples at 15 DAT as influenced by Zn application at various growth stages of *kharif* and *boro* rice.

Treatments	Chl. a (mg/g leaf)			Chl. b (mg/g leaf)			Total Chlorophyll (mg/g leaf)		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	0.85b	0.86e	0.85d	1.49b	1.41d	1.45d	2.34b	2.27d	2.31d
T_2	1.13a	1.03b	1.08a	1.89a	1.80ab	1.85ab	3.02a	2.83a	2.93a
T_3	0.92b	0.89de	0.90cd	1.59b	1.54cd	1.56c	2.51b	2.43cd	2.47c
T_4	0.91b	0.93cd	0.92c	1.55b	1.59c	1.57c	2.46b	2.52bc	2.49c
T_5	1.07a	0.97c	1.02b	1.85a	1.68bc	1.77b	2.92a	2.65b	2.79b
T_6	1.10a	1.11a	1.10a	1.89a	1.89a	1.89a	2.99a	3.00a	3.00a
T_7	0.89b	0.93cd	0.91c	1.54b	1.44d	1.49cd	2.43b	2.37cd	2.40cd
T_8	0.91b	0.89de	0.90cd	1.61b	1.55cd	1.58c	2.52b	2.44cd	2.48c

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

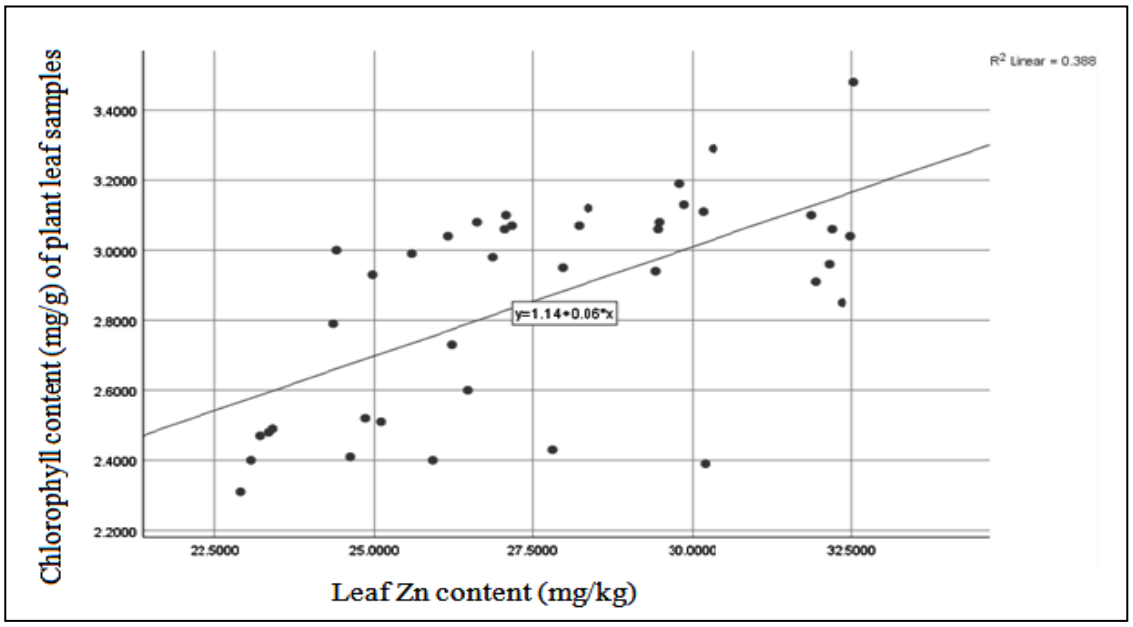

Fig.2.Relationship between leaf chlorophyll content (mg/g) and leaf Zn content (mg/kg) as influenced by Zn application at various growth stages of *kharif* and *boro* rice.

At 15 DAT highest values of chlorophyll a, b and total chlorophyll content were recorded in treatment T_6 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ at the time of flowering) (with values 1.10, 1.89 and 3.00 mg/g for chlorophyll a, b and total chlorophyll content respectively) followed by T_2 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of land preparation) (with values 1.08, 1.85 and 2.93 mg/g for chlorophyll a, b and total chlorophyll content respectively) and T_5 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ after 25 days of transplanting) (with values 1.02, 1.77 and 2.79 mg/g for chlorophyll a, b and total chlorophyll content respectively). Significantly higher chlorophyll content was recorded in these treatments in comparison to control (T_1). Treatment T_2 remained statistically *at par* with treatment T_6 in case of chlorophyll a, b and total chlorophyll content. Lowest value was recorded in control (T_1).

At 30 DAT highest chlorophyll a, b and total chlorophyll content were received for treatment T_5 (with values 1.14, 1.96 and 3.10 mg/g for chlorophyll a, b and total chlorophyll content, respectively) followed by treatment T_3 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half after 25 days of transplanting) (with values 1.14, 1.93 and 3.07 mg/g for chlorophyll a, b and total chlorophyll content respectively) and T_7 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half after 25 days of Transplanting + half $ZnSO_4.7H_2O$ at the time of flowering) (with values 1.12, 1.92 and 3.04 mg/g for chlorophyll a, b and total chlorophyll content respectively). These treatments recorded significantly higher chlorophyll content in comparison to control (T_1). In case of chlorophyll a and b content, treatment T_5 remained statistically *at par* with treatments T_2, T_3, T_6, T_7 whereas in case of total chlorophyll content treatment T_5 remained statistically *at par* with treatments T_3, T_6 and T_7.

At 45 DAT highest chlorophyll a content was recorded in treatment T_7 and T_3 (1.15 mg/g) which remained statistically *at par* with treatments T_2, T_5 and T_6 with values of 1.10, 1.14 and 1.12 mg/g, respectively. Highest chlorophyll b content was recorded in treatment T_5 (1.98 mg/g) which remained statistically *at par* with treatments T_3, T_6 and T_7 with values of 1.92, 1.96 and 1.91 mg/g. Like chlorophyll b content, highest total chlorophyll content was recorded in treatment T_5 (3.12 mg/g) which remained statistically *at par* with treatments T_3, T_5 and T_6 with values of 3.07, 3.08 and 3.06 mg/g, respectively. Lowest chlorophyll content was recorded in control (T_1).

At the time of flowering highest chlorophyll a content was recorded for the treatment T_8 (NPK + 0.30% nano Zn oxide spray at the time of flowering and post flowering) (1.25 mg/g) which remained statistically different from other treatments, followed by T_4 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of flowering) with value of 1.20 mg/g. Similar results were recorded for chlorophyll b and total chlorophyll content with values of 2.04 and 3.29 mg/g. In case of chlorophyll b, treatment T_8 remained statistically *at par* with treatments T_6 and T_7 with values of 2.02 and 1.96 mg/g whereas treatment T_8 remained statistically *at par* with treatment T_6 with value of 3.19 mg/g in case of total chlorophyll content. The above results suggest that Zn applications either as soil or foliar application increased the total chlorophyll content in comparison to control.

At the time of post-flowering highest Chlorophyll a, b and total chlorophyll content were recorded for the treatment T_8 with values of 1.27, 2.20 and 3.48 mg/g respectively. Lowest chlorophyll content was recorded in control (T_1). Treatment T_8 remained statistically different from all other treatments in case of chlorophyll b and total chlorophyll content. In case of chlorophyll a, treatment T_8 was statistically *at par* with treatment T_4 with value of 1.18 mg/g. In the present study, in all tested plant species chlorophyll b content was higher than that of chlorophyll a. Pooled data showed that foliar Zn fertilization at the time of flowering and post flowering resulted an increase in chlorophyll a and b content by a factor of 42.70% and 46.67% respectively in comparison to control at the time of post-flowering.

Above results showed that Zn application significantly increased chlorophyll content in comparison to plots receiving no Zn application. Less chlorophyll content was observed in control plot.

Abbott (1999) reported that chlorophyll is an indicator of plant health, which determines the colour and appearance. It is the photosynthetic rate which determines the growth and yield of crop, which in turn is dependent on chlorophyll contents. Researchers reported that Zn

deficiency decreased chlorophyll level and photosynthetic activity of plants (Hu & Sparks, 1991; Cakmak, 2000; Chen et al., 2008; Fu et al., 2014). Aravind & Prasad (2004) reported that in chlorophyll formation Zn is involved through regulation of nutrients homeostasis in cytoplasm. Grewal et al. (1997) observed that the application of Zn increased chlorophyll content i.e., chlorophyll a, b as compared to control. Chlorophyll structure gets deformed due to Zn deficiency and efficiency of photosynthetic enzymes such as carbonicanhydrase, fructose-1,6-bisphosphase and ribulose 1,5-bisphosphate carboxylase/ oxygenase get limited (Sasaki et al., 1998; Chen et al., 2008). Zn deficiency caused significant decrease in chlorophyll content and photosynthetic rate (Li et al., 1999). Photosynthesis rate increased with Zn application (Dubey, 2005). Some enzymes which are required in the chlorophyll biosynthetic pathways are triggered by Zn (Ayad et al., 2010). Biosynthesis of chlorophyll gets enhanced by foliar or soil application of Zn (Mousavi, 2011) which is important for photosynthesis process. Arif et al. (2012) reported an increase in chlorophyll a and b content by a factor of 43% and 41%, respectively by Zn fertilization in comparison to control. Shitole & Dhumal (2012) reported that micronutrient application caused significant increase in photosynthetic pigments like chl a, b due to increase in secondary metabolic processes. Kabeya & Shankar (2013) reported similar results in their studies on effect of different levels of Zn on growth and uptake ability in rice Zn contrast lines. Qiao et al. (2014) reported that carbonic anhydrase activity increased when Zn is applied on foliage in rice plant leaves, which in turn increased photosynthesis. Carbonic anhydrase is a Zn containing enzyme which is involved in photosynthesis. Hussain (2015) reported that SPAD (an index of chlorophyll content) values significantly increased with application of Zn levels over control. Application of 6 kg Zn/ ha significantly increased SPAD (an index of chlorophyll content) values over control. Zn influence different metabolic pathways hence its deficiency causes reduction in the rate of photosynthesis (Ilyas et al., 2015). Mathpal et al. (2015) reported that Zn application increased chlorophyll content in rice leaves. Kandoliya et al. (2018) reported significant effect of Zn and Fe application (both soil and foliar application) on leaf chlorophyll content of wheat in calcareous soil of Saurashtra region.

Leaf Zn content at 15, 30, and 45 DAT, flowering and post-flowering ofrice

Rice leaf samples were analyzed for Zn content at 15DAT, 30DAT, 45DAT, at the time of flowering and post-flowering during 2017, 2018. Data related to Zn content (mg/kg) in rice leaf samples recorded at various growth stages during 2017, 2018 and pooled of two years is presented in Table 7. Pooled data showed that both soil as well as foliar Zn fertilization had significant impact on Zn concentration in rice leaves collected at various growth stages ($P < 0.05$). Zn addition significantly increased Zn content in rice leaves over control (T_1).

Leaf Zn content (mg/kg) at 15 DAT ranged from 22.90 to 24.97 mg/kg. Highest leaf Zn content (24.97 mg/kg) was recorded in treatment T_2 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of land preparation) which remained statistically different from other treatments, followed by treatments T_6 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ at the time of flowering) and T_5 (NPK + Zn @ 5 kg Zn/ha i.e. 2.5 kg $ZnSO_4.7H_2O$/ ha at the time of land preparation + 2.5 kg $ZnSO_4.7H_2O$/ha after 25 days of transplanting) with values of 24.42 and 24.37 mg/kg respectively. Zn application resulted significant increase in leaf Zn content in comparison to control (T_1). Lowest leaf Zn content (22.90 mg/kg) was received for control (T_1) treatment.

Leaf Zn content (mg/kg) at 30 DAT ranged from 24.63 to 27.18 mg/kg. Highest leaf Zn content (27.18 mg/kg) was recorded in treatment T_3 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at 25 DAT) followed by treatments T_5, T_2, T_7 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half dose after 25 days of Transplanting + half $ZnSO_4.7H_2O$ at the time of flowering) and T_6 with values of 27.09, 26.86, 26.15 and 25.57 mg/kg respectively. Treatment T_3 remained statistically *at par* with treatments T_2 and T_5. Lowest value was received for control (T_1) (24.63 mg/kg). Zn applied treatments resulted significantly higher leaf Zn content in comparison to treatments receiving no Zn application.

Leaf Zn content (mg/kg) at 45 DAT ranged from 25.89 to 28.36 mg/kg. Highest value (28.36 mg/kg) was received for the treatment T_5 followed by treatments T_3, T_2, T_7 and T_6 with values of 28.21, 27.96, 27.07 and 26.62 mg/kg respectively. T_5 remained statistically *at par* with treatments T_2 and T_3. Lowest leaf Zn content (25.89 mg/kg) was recorded in control (T_1).

TABLE 3. Chlorophyll content (mg/g) of plant leaf samples at 30 DAT as influenced by Zn application at various growth stages of *kharif* and boro rice

Treatments	Chl. a (mg/g leaf)			Chl. b (mg/g leaf)			Total Chlorophyll (mg/g leaf)		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	0.86c	0.90b	0.88d	1.54b	1.53b	1.53b	2.40b	2.43b	2.41b
T_2	1.13a	1.06a	1.10b	1.90a	1.86a	1.88a	3.03a	2.93a	2.98a
T_3	1.17a	1.12a	1.14a	1.94a	1.92a	1.93a	3.11a	3.04a	3.07a
T_4	0.92c	0.92b	0.92c	1.60b	1.60b	1.60b	2.52b	2.51b	2.52b
T_5	1.16a	1.12a	1.14a	1.97a	1.96a	1.96a	3.13a	3.08a	3.10a
T_6	1.07b	1.11a	1.09b	1.91a	1.89a	1.90a	2.98a	3.00a	2.99a
T_7	1.18a	1.07a	1.12ab	1.94a	1.90a	1.92a	3.12a	2.97a	3.04a
T_8	0.92c	0.91b	0.91cd	1.61b	1.60b	1.60b	2.52b	2.51b	2.51b

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

TABLE 4. Chlorophyll content (mg/g) of plant leaf samples at 45 DAT as influenced by Zn application at various growth stages of *kharif* and *boro* rice

Treatments	Chl. a (mg/g leaf)			Chl. b (mg/g leaf)			Total Chlorophyll (mg/g leaf)		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	0.89c	0.90b	0.89c	1.54b	1.46d	1.50d	2.43c	2.36d	2.40c
T_2	1.18a	1.02ab	1.10a	1.94a	1.76abc	1.85ab	3.12ab	2.78abc	2.95a
T_3	1.18a	1.12a	1.15a	1.93a	1.92ab	1.92a	3.10ab	3.04ab	3.07a
T_4	0.93c	0.99ab	0.96bc	1.59b	1.69bcd	1.64c	2.52c	2.68bcd	2.60b
T_5	1.16a	1.12a	1.14a	1.99a	1.96a	1.98a	3.15a	3.09a	3.12a
T_6	1.12ab	1.12a	1.12a	1.97a	1.95a	1.96a	3.08ab	3.07a	3.08a
T_7	1.18a	1.13a	1.15a	1.96a	1.86ab	1.91a	3.13ab	2.99ab	3.06a
T_8	1.04b	0.95b	0.99b	1.86a	1.63cd	1.74bc	2.89b	2.57cd	2.73b

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

TABLE 5. Chlorophyll content (mg/g) of plant leaf samples at flowering as influenced by Zn application at various growth stages of *kharif* and *boro* rice

Treatments	Chl. a (mg/g leaf)			Chl. b (mg/g leaf)			Total Chlorophyll (mg/g leaf)		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	0.92d	0.89d	0.90e	1.58d	1.48e	1.53e	2.50c	2.36e	2.43e
T_2	1.13c	1.03c	1.08d	1.92bc	1.79d	1.85d	3.05b	2.82d	2.94d
T_3	1.17bc	1.12b	1.14c	1.94abc	1.93abc	1.93bcd	3.11b	3.05abc	3.08bc
T_4	1.23ab	1.17b	1.20b	1.84c	1.99a	1.92cd	3.07b	3.16ab	3.11bc
T_5	1.16bc	1.14b	1.15c	1.99abc	1.82cd	1.91cd	3.15b	2.96cd	3.06c
T_6	1.22ab	1.13b	1.17bc	2.05ab	1.98ab	2.02ab	3.27ab	3.11abc	3.19ab
T_7	1.20abc	1.14b	1.17bc	2.07ab	1.86bcd	1.96abc	3.26ab	3.00bc	3.13bc
T_8	1.26a	1.24a	1.25a	2.12a	1.96ab	2.04a	3.37a	3.20a	3.29a

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

TABLE 6. Chlorophyll content (mg/g) of plant leaf samples at post-flowering as influenced by Zn application at various growth stages of *kharif* and *boro* rice

Treatments	Chl. a (mg/g leaf)			Chl. b (mg/g leaf)			Total Chlorophyll (mg/g leaf)		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	0.91c	0.87d	0.89d	1.52d	1.48d	1.50c	2.43d	2.35c	2.39c
T_2	1.13b	0.92cd	1.03c	1.98b	1.67bcd	1.82b	3.11bc	2.59bc	2.85b
T_3	1.17ab	1.10ab	1.14b	1.75c	1.89abc	1.82b	2.92c	2.99ab	2.96b
T_4	1.24ab	1.11ab	1.18b	2.02b	1.84bc	1.93b	3.26b	2.95b	3.10b
T_5	1.18ab	1.06bc	1.12bc	1.88bc	1.97ab	1.93b	3.05bc	3.04ab	3.04b
T_6	1.20ab	1.05bc	1.13bc	2.07ab	1.81bc	1.94b	3.27b	2.86b	3.06b
T_7	1.23ab	0.97bcd	1.10bc	2.02b	1.60cd	1.81b	3.25b	2.57bc	2.91b
T_8	1.30a	1.25a	1.27a	2.24a	2.17a	2.20a	3.54a	3.42a	3.48a

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

TABLE 7. Analysis of Zn content (mg/kg) in plant leaf samples at 15 DAT, 30 DAT, 45 DAT, flowering and post-flowering as influenced by Zn application at various growth stages of *kharif* and *boro*rice

Treat.	Total Zn (mg/kg) 15 DAT			Total Zn (mg/kg) 30 DAT			Total Zn (mg/kg) 45DAT			Total Zn (mg/kg) F			Total Zn (mg/kg) PF		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	22.24d	23.56b	22.90d	23.74c	25.53e	24.63d	25.36c	26.41e	25.89d	27.32b	28.28c	27.80d	30.74b	29.64c	30.19d
T_2	24.37a	25.57a	24.97a	26.37a	27.35bc	26.86a	27.80a	28.12bc	27.96a	28.83a	29.97b	29.40c	31.80a	32.87a	32.34ab
T_3	22.43cd	23.96b	23.20cd	26.42a	27.94a	27.18a	27.78a	28.65ab	28.21a	28.91a	30.01b	29.46c	31.74a	32.54ab	32.14abc
T_4	22.80c	23.98b	23.39c	24.61b	25.11e	24.86d	26.57b	26.39e	26.48c	29.05a	31.28a	30.16ab	31.70a	32.03b	31.86c
T_5	23.53b	25.21a	24.37b	26.39a	27.80ab	27.09a	27.80a	28.91a	28.36a	28.94a	29.94b	29.44c	31.94a	33.03a	32.48a
T_6	23.44b	25.40a	24.42b	24.76b	26.39d	25.57c	25.89c	27.36d	26.62c	29.13a	30.44b	29.79bc	31.79a	32.58ab	32.18abc
T_7	22.40cd	23.71b	23.06cd	25.39b	26.91c	26.15b	26.68b	27.45cd	27.07b	29.12a	30.61ab	29.86abc	31.72a	32.15b	31.93bc
T_8	22.70cd	23.97b	23.33c	24.90b	25.31e	25.10d	25.60c	26.81de	26.21cd	29.42a	31.21a	30.32a	31.99a	33.08a	32.53a

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

Leaf Zn content ranged from 27.80 to 30.32 mg/kg at the time of flowering. Highest value (30.32 mg/kg) was received for treatment T_8 (NPK + 0.30% nano Zn oxide spray at the time of flowering and post flowering) which remained statistically *at par* with treatments T_4 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ at the time of flowering) and T_7 with values of 30.16 and 29.86 mg/kg, respectively. Leaf Zn concentration at the time of flowering were found in the sequence: T_8> T_4> T_7> T_6> T_3> T_5> T_2> T_1. Lowest value (27.80 mg/kg) was recorded in control treatment (T_1). Above results suggest that Zn applications either as soil or foliar application increased leaf Zn content in comparison to control.

At the time of post-flowering highest leaf Zn content was achieved with the treatment receiving 0.30% nano ZnO foliar spray at the time of flowering and post-flowering along with recommended NPK (T_8) (32.53 mg/kg). Lowest leaf Zn content (30.19 mg/kg) was recorded in control (T_1). T_8 remained statistically *at par* with treatments T_2, T_3, T_5 and T_6 with values of 32.34, 32.14, 32.48 and 32.18 mg/kg respectively. Leaf Zn concentration at the time of post-flowering followed the order: T_8> T_5> T_2> T_6> T_3> T_7> T_4> T_1. Foliar Zn fertilization resulted significant increase in leaf Zn content compared to soil application. Increase in leaf Zn concentration with soil Zn fertilization can be explained by considering the fact that soil $ZnSO_4.7H_2O$ application increases available Zn content in soil which then gets absorbed by plant roots whereas Zn gets directly absorbed by leaf epidermis when applied on foliage. Binding of available Zn content present in soil solution with soil components can be avoided by foliar spray. Split application of $ZnSO_4.7H_2O$ performed better in terms of increasing leaf Zn content over single soil application. Hence higher leaf Zn content was recorded in treatments T_5, T_6, T_7 over treatments T_2, T_3, T_4, respectively. Higher soil Zn content can be achieved with split application of $ZnSO_4.7H_2O$ over single soil application, which might be due to the less residual effect of $ZnSO_4.7H_2O$ compared to chelated Zn fertilizers (Naik & Das, 2007). Under submerged condition, Zn^{+2} dissociated from inorganic $ZnSO_4.7H_2O$ fertilizer precipitates as Zn carbonate ($ZnCO_3$) or Zn sulphide (ZnS), hence lowering the availability of Zn. Hence to maintain available Zn content in soil, split application is preferred over single soil application. Results indicate the effectiveness of foliar Zn fertilization on leaf Zn concentration over soil Zn fertilization. Increase in leaf Zn concentration with foliar Zn application can be explained by considering the fact that ZnO is having low molecular weight, hence can easily penetrate into the rice plant leaves.

Robson (1993) reported that factors such as available Zn content in soil, transfer of Zn to plant root surfaces and interaction between Zn and other nutrients between Zn and other nutrients within the plant or in the soil control the Zn content of plant. Higher efficiency was recorded with 25 kg Zn/ha as $ZnSO_4$ application at the time of transplanting and at the time of tillering compared to the Zn application at the time of transplanting and panicle initiation (Savithri et al., 1999). Khan et al. (2002) reported significant increase in leaf Zn content of rice crop with Zn application at the rate of 5 kg/ha as $ZnSO_4$ before flowering (47.48 mg/kg) in comparison to control (29.66 mg/kg). Zn fertilization significantly increased leaf Zn content after flowering (34.45 mg/kg) over control (23.58 mg/kg). Higher Zn content before flowering might be due to high vigor of plants and greater root activity for better uptake of Zn. Iqbal et al. (2000) reported similar result in their studies. Zn fertilization as $ZnSO_4$ at the rate of 5 kg/ha resulted significant increase in Zn content in rice leaf before flowering and after flowering in comparison to control (T_1) in eight different calcareous soil series of D.I.Khan (Khan et al., 2004). Enhanced Zn concentration in different parts of rice plant with increased Zn application might be due to the higher absorption of Zn from soil solution to rice plants (Fageria et al., 2011). Zn fertilization had positive effect on straw, leaf and grain samples of rice crop (Kabeya & Shankar, 2013). Due to complex soil reaction, Zn uptake through plant's root is limited, hence foliar application appears to be most advantageous method as no such problem arises in case of foliar application (Mabesa et al., 2013). Zn fertilization at the rate of 5 kg/ha as $ZnSO_4.7H_2O$ resulted significant increase in Zn content at different stages of rice crop (Muthukumararaja & Sriramachandrasekharan, 2019). They reported that at tillering stage Zn content ranged from 32.76 to 58.78 mg/kg and at panicle initiation stage its value ranged from 26.93 to 52.53 mg/kg.

Quality parameters

Crude protein content in rice grains

The data regarding crude protein content (%) in rice grain during 2017, 2018 and pooled of two years is presented in Table 8. Protein

is considered to be the most important factor which determines the quality of the grain. In the endosperm of rice grain, protein is considered to be major organic substance contributing 5-12% to the total dry weight of the grain (Chen et al., 2012). As protein is required in a large amount in our bodies, hence grain with better protein content is preferable. Pooled data showed that Zn application significantly affected crude protein content of rice grain ($P < 0.05$). Crude protein content in rice grain was influenced by soil $ZnSO_4.7H_2O$ application as well as foliar 0.30% nano Zn oxide spray. Crude protein content value ranged from 11.37% to 12.95%. Highest crude protein (12.95%) was recorded in treatment T_8 (NPK + 0.30% nano Zn oxide spray at the time of flowering and post flowering) which was 13.90% higher in comparison to control (T_1), followed by treatment T_5 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ after 25 days of transplanting) which contained 12.83% crude protein. Lowest crude protein content (11.37%) was recorded in treatment T_1 (control). Both soil $ZnSO_4.7H_2O$ application and foliar nano Zn oxide spray significantly affected crude protein in rice grain. Treatment T_8 was statistically *at par* with treatments T_5 and T_6 (NPK + Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$, half at the time of land preparation + half $ZnSO_4.7H_2O$ at the time of flowering).

Researchers reported that Zn application significantly increased grain protein content (Biswas et al., 1977; Tandon, 1992; Zeidan et al., 2010; Keram, 2014). For the synthesis of protein and nucleic acid, high Zn concentration is required in meristematic tissues (Brown et al., 1993). Marschner (1995) reported that protein synthesis is reduced when Zn deficiency is observed in plants. He further reported that Zn is required for the synthesis of tryptophan, which is the precursor for the biosynthesis of indole acetic acid (IAA). Zn application resulted enhanced tryptophan content. % Protein content in coriander seeds was increased significantly with Zn application (Singh, 1998). Grain rice quality enhanced with Zn fertilization (Li et al., 1999). Thalooth et al. (2006) reported that grain protein content of mungbean increased with $ZnSO_4$ application. Broadley et al. (2007) reported that several physiological functions in plants such as synthesis, integrity and functioning of proteins are influenced by Zn. Hence concentration of protein in rice grain is increased with Zn application. Researchers reported positive and close relationship between Zn and protein concentration in wheat (Morgounov et al., 2007) and gram seed (Roy et al., 2014). Ranjbar & Bahmaniar (2007) reported that Zn application significantly increased grain protein content. Alloway (2008) reported that in Zn deficient plants protein content is reduced. Protein content is increased after administration of Zn for 48 or 72 hrs. Zn application along with N application increased crude protein content of grain. Khan et al. (2009) reported that grain protein content increased significantly with application of Zn along with N. Researchers (Seadh et al., 2009; El-Habbasha et al., 2015) reported positive effect of foliar Zn application on increasing grain protein content. Cakmak et al. (2010) reported that there existed positive correlation between grain Zn concentration and grain protein content. Morshedi & Farahbakhshb (2010) also reported that grain protein content of wheat increased with Zn application. Marschner (2012) reported that biosynthesis of proteins gets impaired due to Zn deficiency. Cation-exchange capacity of the roots gets enhanced by Zn, which enhances absorption of essential nutrients such as N which is responsible for higher protein content (Siavoshi & Laware, 2013). Humaira et al. (2015) reported that Zn-superoxide dismutase enzymatic activity is enhanced with Zn application. Due to this, more protein is produced. Obata et al. (1999) reported similar result in their studies. Khattak et al. (2015) reported that regardless of the method of Zn application i.e. both soil as well as foliar Zn application significantly increased protein content in wheat grain ($P < 0.05$). They further reported that grain protein content can be increased either by low level of soil Zn application along with higher level of foliar Zn application or by higher level of soil Zn application along with low level of foliar Zn spray. The increased protein content due to Zn fertilization might be explained by considering the mechanism that Zn application causes reduction in RNA, deformation and then reduction in ribosome. Mathpal et al. (2015) reported that soil application of Zn increased grain protein content in comparison to control. Drostkar et al. (2016) reported that nanofertilizer application increased grain protein content of chickpea dramatically. Afzal et al. (2017) reported that Zn application both as soil and foliar spray significantly increased wheat grain protein content in comparison to control (9.20%). Crude protein content in both bran and flour of wheat increased significantly with Zn application (Akgun et al., 2017). El-Dahshouri et al. (2017) reported that

grain protein content increased significantly with foliar Zn application. Kumar et al. (2017) reported that basal application of Zn as ZnO at the rate of 5 kg/ha increased protein content in rice grain and straw in comparison to control. Similar results were confirmed by Khanna et al. (2002); Singh et al. (2004); Tripathi & Tripathi (2004). Fergany (2018) reported that grain crude protein was significantly affected when Zn was applied on foliage. Hussain et al. (2018) reported that Zn application increased grain protein content (8 to 11%) over control. Kandoliya et al. (2018) reported that soil Zn application influenced wheat grain protein content.Foliar Zn spray was found to be the most effective way to increase grain protein content (Kamboj & Mathpal, 2019). Roy et al. (2019) reported a positive linear relation (R^2 = 0.0774) between grain Zn content (g) and grain protein content (µg/g).

Amylose content in rice grains

The data regarding amylose content (%) in rice grain during 2017, 2018 and pooled of two years is presented in Table 8. Pooled data showed that amylose content in rice grain did not vary significantly with Zn application at various growth stages of rice crop. However, its value in rice grain ranged from 21.15% to 22.03%. Bhattacharya et al. (1982) reported the classification of rice depending on amylose content. Rice is waxy, low, intermediate and high when amylose content is 0-2%, 10-20%, 20-25% and > 25%. In the present study, rice grains were found to contain intermediate amylose content. De (1999) reported that amylose content of rice grain is related to its cooking and eating quality. High amylose content of rice grain is beneficial for health. Ali et al. (2014) reported that kernel amylose contents were not affected with Zn fertilization. Panhwar et al. (2015) reported that micronutrient application has no significant effect on amylose content in rice grain. Chatterjee & Das (2018) reported that in case of unpolished rice (brown rice) amylose content was found to be less in comparison to polished rice (white rice). This might be due to the milling of unpolished rice in order to get polished rice. In this process bran is removed and concentration of starch is higher in endosperm in comparison to bran.

Starch content inrice grains

The major dietary source of carbohydrate is starch. 70% of starch has amorphous region consisting of amylose and branching points of amylopectin molecules. The remaining 30% is having crystalline region consisting of outer chains of amylopectin (Reddy & Bhotmange, 2013). The data regarding starch content (%) in rice grain during 2017, 2018 and pooled of two years is presented in Table 8. Pooled data showed that starch content in rice grain did not vary significantly with treatments receiving Zn application at various growth stages of rice crop at 5% level of probability. However, the value of starch content in rice grain varied from 79.26% to 80.27%.

TABLE 8. Quality parameters (% crude protein, % amylose and % starch) as influenced by Zn application at various growth stages of *kharif* and *boro* rice

Treatments	% Crude protein			% Amylose			% Starch		
	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled	Kharif (2017)	Boro (2018)	Pooled
T_1	10.97^{b}	11.78^{b}	11.37^{c}	22.45^{a}	20.73^{a}	21.59^{a}	80.64^{ab}	77.89^{a}	79.26^{a}
T_2	11.67^{ab}	12.13^{ab}	11.90^{abc}	21.70^{a}	22.25^{a}	21.97^{a}	82.00^{ab}	77.92^{a}	79.96^{a}
T_3	11.78^{ab}	11.90^{b}	11.84^{bc}	21.46^{a}	21.30^{a}	21.38^{a}	81.13^{ab}	78.58^{a}	79.85^{a}
T_4	12.48^{a}	11.90^{b}	12.19^{abc}	22.18^{a}	21.26^{a}	21.72^{a}	81.45^{ab}	79.09^{a}	80.27^{a}
T_5	12.72^{a}	12.95^{ab}	12.83^{ab}	22.42^{a}	21.64^{a}	22.03^{a}	81.10^{ab}	78.52^{a}	79.81^{a}
T_6	12.48^{a}	12.25^{ab}	12.37^{abc}	22.28^{a}	20.53^{a}	21.41^{a}	82.15^{a}	76.85^{a}	79.50^{a}
T_7	12.13^{ab}	12.25^{ab}	12.19^{abc}	21.72^{a}	20.95^{a}	21.34^{a}	81.93^{ab}	77.38^{a}	79.65^{a}
T_8	12.60^{a}	13.30^{a}	12.95^{a}	21.33^{a}	20.97^{a}	21.15^{a}	80.36^{b}	77.34^{a}	78.85^{a}

Treatments: T_1: (Control, no Zn application); T_2: [Zn @ 5 kg Zn/ha as $ZnSO_4.7H_2O$ (Basal)]; T_3: [Zn as $ZnSO_4.7H_2O$ at 25 DAT]; T_4: [Zn as $ZnSO_4.7H_2O$ at Flowering]; T_5: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at 25 DAT]; T_6: [Zn as $ZnSO_4.7H_2O$, half dose as basal + half dose at Flowering]; T_7: [Zn as $ZnSO_4.7H_2O$, half dose at 25 DAT + half dose at Flowering]; T_8: 0.03% Nano-ZnO spray at the time of flowering and post- flowering.

Recommended dose of N, P_2O_5 and K_2O @ NPK (80-40-40) kg/ha were applied through urea and fertilizer grade N:P:K :: 10:26:26. a to h means with the values followed by the same high script in each column are not significant at 5% probability level ($P \leq 0.05$).

Barak & Helmke (1993), Alloway (2004) reported that Zn is involved in starch formation. Rajub (1999) reported that with Zn application starch content in rice grain varied non-significantly. Khan et al. (2004) reported that Zn application had no significant effect on starch content of paddy. Sharma et al. (2016) reported Zn promotes starch formation.

Conclusion

On the basis of present research it can be said that foliar applications of nano-ZnO and soil $ZnSO_4.7H_2O$ application manipulate the growth of rice. In this study, particular attention has been given to Zn concentration in rice leaves collected at various growth stages, chlorophyll content, plant height and quality of rice grain as these factors indicate the effectiveness of soil as well as foliar Zn fertilization. Results indicated that foliar Zn fertilization can improve bioavailability of Zn in rice grain which is evidenced by significant increase in plant height, chlorophyll and leaf Zn content over soil Zn application. Quality of rice grain was improved with foliar Zn spray. In conclusion it can be said that foliar Zn fertilization appears to be an effective agronomic practice over soil Zn fertilization in order to increase leaf Zn concentration, chlorophyll content, plant height and quality of rice grain. Above results explain the higher efficiency of foliar Zn application over soil application. Data regarding plant height, quality of rice grain, chlorophyll and leaf Zn content suggested that 0.30% foliar nano-ZnO spray at the time of flowering and post flowering was the best dose.

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