

Research Article

Prospective of Use of Phosphorus and Zinc at Tillering and Booting Stages of Rice

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Abstract | Phytic acid is substance that stores most of phosphorus in seeds of many cereals. It is a strong chelating agent which can chelate essential nutrients like zinc, iron, calcium and magnesium resulting in mineral deficiency in masses of developing countries. Low phytic acids is therefore a primary factor to enhance availability of these nutrient and combat phytic acid related issues. In this study, the most suitable crop stage and combination of zinc and phosphorus application was investigated to decrease phytin content and enhance zinc bioavailability in rice grain. Three level of phosphorus fertilizer viz. 60, 90, 120 kg ha⁻¹ were employed with 5 and 10 kg ha⁻¹ of zinc fertilizer. Treatment applied with the recommended dose of nitrogen, phosphorus and potassium was taken as control. Paddy yield data were collected and rice grain was analysed for phosphorus, zinc and phytic acid contents. Findings showed that maximum paddy yield (4.80 t ha⁻¹) was found where P and Zn were applied 120 and 5 kg ha⁻¹ respectively with maximum P (0.55%), Zn (13.8 mg kg⁻¹) and phytic acid (0.38%) contents. Lowest phytic acid contents (0.32%) were observed in control treatment. The results depicted that yield, P and Zn contents of rice grain improved significantly due to synergistic effect of P and Zn fertilizers applied at different growth stages. The phytic acid contents in rice grains remained within safe limit in all treatments but lower grain phytic acid have been observed where only recommended dose of NPK fertilizers were used.

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Introduction

Mineral fertilizer of phosphorus provides high crop yield. Global phosphorus fertilizer consumption has raised from 4.6 million tonnes in 1961 to an estimated 22–27 million tonnes phosphorus per year by 2050 (Bindraban *et al.*, 2020). Phosphorus is a primary nutrient that is required by plants in larger quantities for plant structure and functions i.e., cell division, root development, flowering, fruit and seed formation. However, usually it is less available to plants, which affects plant productivity. P is redistributed in source and sink organs of plants to enhance its utility and homeostasis. In case of P deficiency, P is relocated from older leaves to younger leaves while during reproductive season, P is redistributed from vegetative parts to seeds and converts into phytic acid in cereals (Perera *et al.*, 2019). P can be remobilized up to 25–30% from vegetative organs to the grain (Xie *et al.*, 2018).

Phytic acid (PA) is almost impenetrable in human body and most occurring substance of phosphorus in seeds. Genetic modification can reduce level of phytic acid in rice and enhance nutritional value of crop (Larson *et al.*, 2000). PA stores in seeds, nuts, legumes and grains in fairly high quantity on weight basis, about 1–5% of human needs. About 70% of its weight is made of P. It can attach iron, zinc and calcium along with other ions (Garcia-Esteva *et al.*, 1999; Raboy *et al.*, 2000; Erdal *et al.*, 2002; Mate and Radomir, 2002; Gallaher, 2012; Nissar *et al.*, 2017; Perera *et al.*, 2018), which can cause deficiency of micronutrients in food due to less diversity in available food. PA is present up to 5% in some brans increasing its chances to be in food chain. Marginal availability of micronutrients enhances due to the presence of PA. It converts into other inositol phosphates during metabolic processes in the gut, IP5 and IP6 fixes nutrients. PA is present from 4–7 mg g⁻¹ in rice, oat, barley and rye (Garcia-Esteva *et al.*, 1999).

About 2 million people suffer from micronutrient deficiency (FAO, 2014), moreover, micronutrient deficiency is more prevalent in developing states (IFPRI, 2016). Nutrient scarcity of zinc and iron, due to tendency of phytic acid to bind them, make them unavailable to human digestive system. These nutrients are indispensable for proper development and growth of human body. Cooking can lessen content of phytic acid in rice, peanut, soybean, sunflower and pigeon pea

seeds but it does not enhance availability of nutrients as expected (Mahesh *et al.*, 2015; Kumar *et al.*, 2010).

Malnutrition has been a serious problem of man since ages. About 870 million of world population are undernourished which made 13% of total population of the globe (WHO, 2014). This phenomenon has deteriorated quality of human life and its work quality badly in the recent years as well (White and Brown, 2010). Need of micronutrients is not very high, still a big ratio of women and children are suffering from its deficiency (Ramakrishna *et al.*, 2006). Other than soil fertility status, phytate is a potential anti-nutritional factor which decreases the bioavailability of micronutrients (Shi *et al.*, 2003).

Noulas *et al.* (2018) describes effective purpose of zinc in plant as in photosynthesis, membrane structure, protein synthesis and disease and drought tolerance. Though it is micronutrient still, its deficiency can cause challenging environment to market value of various crops. There is ever increasing need of extra food due to population explosion and role of zinc in this regard cannot be neglected. Sandy, saline and calcareous soils are reported to be zinc deficient and agricultural products taken from them do not satisfy human need of zinc for proper body functioning. Broadley *et al.* (2007) endorses essentiality of zinc as a nutrient for growth of plants as it is part of many proteins in plants. Essentiality of zinc has been proven in maize, barley and sunflower in start of 20th century, as its deficiency causes many warning signs during plant life. Zinc deficit is very prevalent in high pH soils (Shahid *et al.*, 2016). Kaya *et al.* (2009) described that zinc application in soil gave a boost to zinc content in seed of chickpea. It also had a negative relation with PA and seed P. Proportion of high phytate food determines the bio-availability of Zinc in cereals and legumes. High ratios of phytate: zinc i.e., 15:1 provides less zinc (<15%) (Allen *et al.*, 2006).

Rice (*Oryza sativa* L.) holds a position of main staple diet of about half population of the globe. Rice contributes 21% and 15% per capita of dietary energy and protein. Rice is sensitive to zinc deficiency, and zinc is a vital micronutrient limiting rice production in term of quality and quantity (Yadi *et al.*, 2012). Rice is significant food crop in Pakistan after wheat. It is an important cash crop after cotton in terms of export (Pakistan Economic Survey, 2017–18). Keeping in view the said scenario, this experiment was performed

to observe prospective use of phosphorus and zinc at tillering and booting stages of rice.

Materials and Methods

Site description

This experiment was executed at Soil Chemistry Section, ISCES, AARI, Faisalabad, Punjab, Pakistan. Faisalabad is located at 185.6 meters above sea level. Faisalabad's climate is semi-arid, summer is hot and humid and winter is dry and cool. The temperatures average from 40.5°C to 26.9°C in June and 19.4°C to 4.1°C in January. Annual rainfall averages approximately at 375 millimetres and highly seasonal (Agromet Bulletin, 2017). The soil of experiment is mainly sandy clay loam having pH 8.3, EC 1.26 dSm⁻¹, organic carbon 3.7g kg⁻¹, organic matter 0.70%, available P 8.3 mg kg⁻¹ and available potash 200 mg kg⁻¹ at surface soil (0-15 cm) while subsurface soil contained 8.1 pH, 1.20 dSm⁻¹ EC, 3.4g kg⁻¹ organic carbon, 0.67% organic matter, 8.1 mg kg⁻¹ available P and 186 mg kg⁻¹ available potash (Table 1). Weather data for experimental season is given in Figure 1.

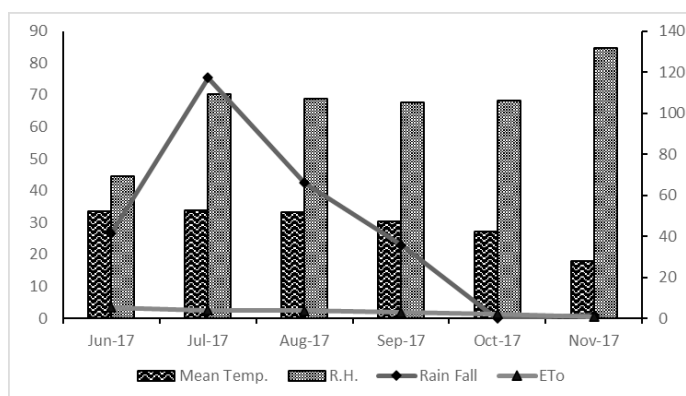


Figure 1: Mean temperature, relative humidity, rainfall and evapotranspiration for growing season of rice.

Soil characteristics

Experiment design: Seven treatments were employed with three repeats by using split plot statistical model in a permanent layout setup.

The plot size was 5m*7m (35m²). Rice was studied at two growth stages i.e., tillering and booting. Growth stages were allotted in main plots while treatments were allotted in sub plots.

Approved dose of NPK viz. 150-90-60 kg ha⁻¹ was applied according to treatment plan using Urea (in two splits), diammonium phosphate and sulphate of potash as basal dose. 1st half of Nitrogen was added as

basal and second dose was applied at panicle initiation stage. Zn was applied at active tillering and booting stage.

Cultivation technology

Rice seedlings were collected from nursery, already grown at farm area of soil chemistry section forty-five days ago, transplanting was done in mid-July. Rice transplanting was done manually in standing water. Cultivation practices were done when required. Rice was harvested in start of November.

Rice sampling and analysis

When the rice plants had reached at maturity, rice plants were harvested. Later, its grain and straw were weighed. Rice grain were dried in air circulating oven at 60°C and crushed with grinding machine and preserved in polythene bags for later use. Paddy samples were analyzed for P, Zn, total phytin, fiber and protein content.

Table 1: Initial soil physico-chemical properties.

| Soil property | Units | Depth (cm) | |
|---------------|---------------------|------------|-------|
| | | 0-15 | 15-30 |
| OC | g kg ⁻¹ | 3.7 | 3.4 |
| EC | dS m ⁻¹ | 1.26 | 1.20 |
| pH | - | 8.3 | 8.1 |
| OM | % | 0.70 | 0.67 |
| Available P | mg kg ⁻¹ | 8.3 | 8.1 |
| Available K | mg kg ⁻¹ | 200 | 186 |

Table 2: Description of experimental treatments.

| Treatment |
|---|
| T ₁ : Control (RD NPK) |
| T ₂ : RD NK+ P @ 60 kg ha ⁻¹ + Zn@ 5 kg ha ⁻¹ |
| T ₃ : RD NK+ P @ 90 kg ha ⁻¹ + Zn @ 5 kg ha ⁻¹ |
| T ₄ : RD NK+ P @ 120 kg ha ⁻¹ + Zn @ 5 kg ha ⁻¹ |
| T ₅ : RD NK+ P @ 60 kg ha ⁻¹ + Zn @ 10 kg ha ⁻¹ |
| T ₆ : RD NK+ P @ 90 kg ha ⁻¹ + Zn @ 10 kg ha ⁻¹ |
| T ₇ : RD NK+ P @ 120 kg ha ⁻¹ + Zn @ 10 kg ha ⁻¹ |

Soil sampling and analysis

Soil samples were taken (0-30 cm) at sowing and harvesting, which were later air dried, grinded and sieved (2mm Sieve) before the further use (Carter, 1993). Soil Texture was determined by Gee and Bauder (1982) method while chemical properties EC and pH was determined using method by (Page et al., 1982). Soil organic carbon was analysed by

Potassium Dichromate Method (Walkley, 1947), soil available P and K extraction was done with 0.5 M NaHCO₃ (Olsen P blue method) and 1 N NH₄OAc, respectively.

Statistical analysis

The data collected was subject to ANOVA using split plot design with three replications. Mean comparison was employed according to LSD test ($P \leq 0.05$). All the statistical analysis was carried out by Statistical 8.1 software (Steel *et al.*, 1997).

Results and Discussions

Growth attributes of rice

The data regarding the impact of Zn and P on rice growth parameters is given in Table 3. Greater plant height found in plot receiving P @ 90 kg ha⁻¹ as basal fertilizer and 5 kg ha⁻¹ Zn at booting stage while control gave minimum plant height. Same pattern was observed in other growth attributes i.e., number (no.) of tillers and no. of grains per panicle. Zinc application along with NPK fertilizer, as compared to NPK fertilizer alone, enhanced no. of productive tillers, plant height and filled grains per panicle likewise panicle length and 1000 grain weight also observed the same trend (Rahman *et al.*, 2011; Sudha and Stalin, 2015).

Yield attributes of rice

The application of phosphorus and zinc have significant impact at booting and active tillering stage of rice. Results revealed that maximum paddy yield i.e., 4.80 tons ha⁻¹ was found where Zn @ 5 kg ha⁻¹ and P @ 120 kg ha⁻¹ were used in comparison to control plot. Second higher yield of paddy (4.75 tons ha⁻¹) was found from treatment receiving phosphorus (120 kg ha⁻¹) along-with Zn (10 kg ha⁻¹) as illustrated in Figure 2. Maximum paddy yield was obtained at booting stage of zinc application. In case of biomass, maximum biomass was attained where P and Zn was used @ 90 kg ha⁻¹ and 5 kg ha⁻¹ which is not significantly different in two growth stages i.e., tillering and booting as it was 15.83 and 16.14 tons/hac respectively as illustrated in Figure 3.

Higher yield was attained due to better plant morphology and health i.e., more no. of tillers per plant, heavy seeds and more number of grain panicles. Phosphorus application boost grain yield of rice and its interaction with zinc has a significant effect on

rice yield as it is clear from the results (Rahman *et al.*, 2011). Phattarakul *et al.* (2009) gave the same finding, Zinc along with Nitrogen enhance rice yield by 12-25%. Similarly, increase in rice biomass may be due to Zn application that increase the straw yield and its interaction with P increased straw yield as reported by Hossain *et al.* (2009). P fertilization have a tendency to enhance grain and straw yield, and, finally total P in plant tissues (Bindraban *et al.*, 2020). Likewise, Zn application concurrently increased crop production and grain Zn concentration under soil conditions supporting low Zn content (Drissi *et al.*, 2015; Dimkpa *et al.*, 2019).

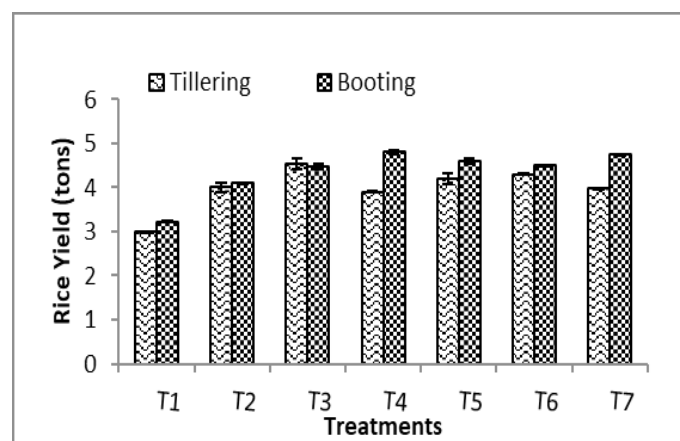


Figure 2: Impact of P and Zn application on rice yield.

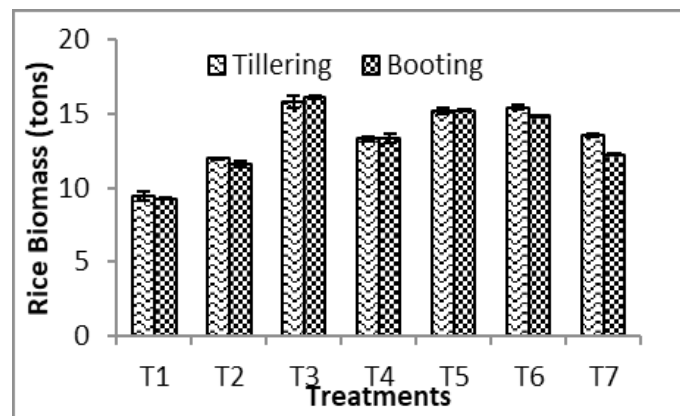


Figure 3: Impact of P and Zn application on rice biomass.

Phytic acid content

Zinc fertilizer application has increased the phytic acid content in rice plant. Figure 4 clearly shows, control plant which had no zinc fertilizer has minimum amount of phytic acid, rest of all treatments had higher concentration of phytic acid, minimum phytic acid was found in T₃ i.e., RD NK+ P @ 90 kg/ha as basal dose and Zn @ 5 kg ha⁻¹ at tillering stage (1.11%) it was non-significant with control plot (1.12%).

Table 3: Impact of P and Zn use on plant growth attributes of rice crop.

| Treatments | Plant height (cm) | | No. of tillers | | No of grains/panicle | |
|----------------|-------------------|-----------|----------------|---------|----------------------|---------|
| | Tillering | Booting | Tillering | Booting | Tillering | Booting |
| T ₁ | 122.00 K | 126.67 J | 14 E | 13 E | 133 H | 139 G |
| T ₂ | 147.67 C | 137.67 H | 17 BCD | 19 ABC | 156 E | 156 DE |
| T ₃ | 149.33 B | 147.00 C | 20 AB | 20 ABC | 163 B | 161 BC |
| T ₄ | 141.33 FG | 151.00 A | 16 DE | 21 A | 152 F | 163 B |
| T ₅ | 140.00 G | 142.67 EF | 17 BCD | 19 ABC | 159 CD | 159 C |
| T ₆ | 144.00 DE | 144.67 D | 18 BCD | 17 BCD | 159 C | 163 B |
| T ₇ | 133.33 I | 147.33 C | 14 E | 17 CD | 151 F | 166 A |
| | 139.52 B | 142.43 A | 17 B | 18 A | 153 B | 158 A |

Means sharing different alphabets are statistically different ($p < 0.05$) using the LSD test.

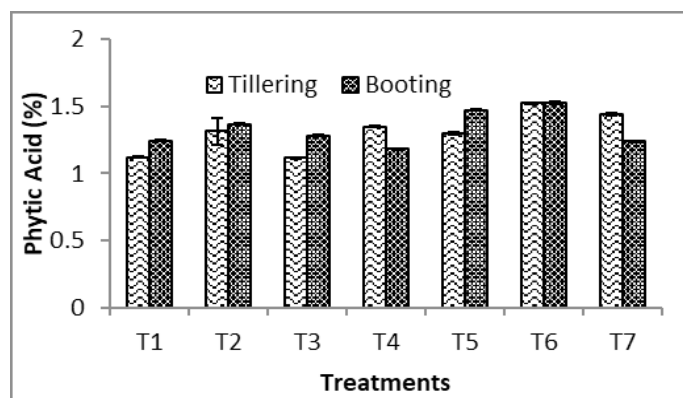


Figure 4: Impact of P and Zn application on rice grain phytic acid (%).

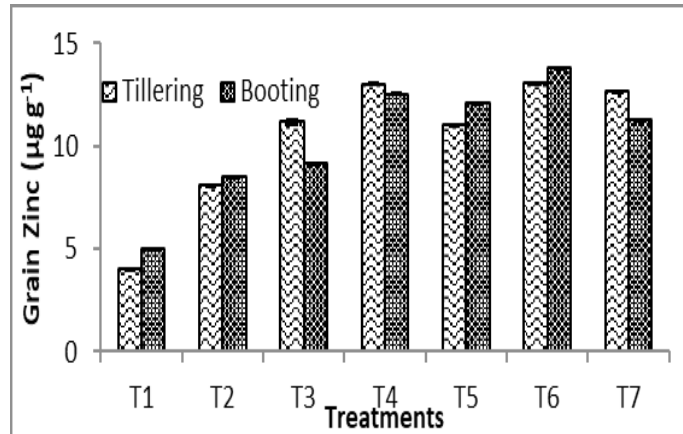


Figure 5: Impact of P and Zn application on rice grain zinc ($\mu\text{g g}^{-1}$).

Kaya *et al.* (2009) wrote that zinc fertilizer application is antagonistically related to phytic acid and seed phosphorus. While study of Wang *et al.* (2011) clarified that phytic acid is mostly present in outer layer of rice kernel, instead of grain. They also found that bran layer has comparatively low concentration of PA as that of other mineral nutrients as Fe, Mn and Mg.

Mineral bioavailability

Phosphorus content (0.55 %) in rice grain was present

in treatment receiving P (90 kg ha^{-1} and 120 kg ha^{-1}) respectively and Zn (10 kg ha^{-1}) at booting stage, as compared to control treatment (0.28%) as illustrated in Figure 5. These results are strengthened by Rehimi *et al.* (2014) and Amanullah *et al.* (2020).

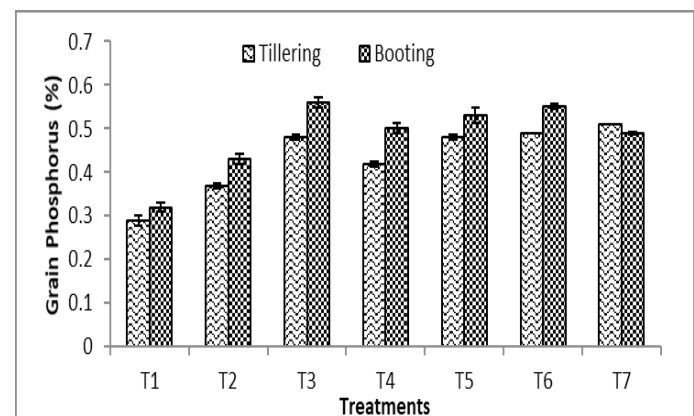


Figure 6: Impact of P and Zn application on rice grain phosphorus (%).

Figure 6 depicts that in case of zinc, highest zinc content (13.83 mg kg^{-1}) accumulated in rice grain was found in treatment receiving 90 kg ha^{-1} P and 10 kg ha^{-1} Zn fertilizer (Zhang *et al.*, 2017). Antagonistic correlation of P fertilizer application on Zn, Ca and Fe concentrations is accompanied with more phytate content which deteriorates dietary value of agricultural products (Bindraban *et al.*, 2020). Soil zinc supplement enhanced zinc content in rice plant. (Fang *et al.*, 2008; Ghoneim, 2016), but Phattarakul *et al.* (2009) reported that zinc foliar application was more beneficial in enhancing grain zinc content, because most of soil applied zinc is accumulated in straw and did not reach the grain.

Interaction between phosphorus and zinc in rice

Phosphorus and zinc interaction showed that zinc content increased with increasing Phosphorus

concentration in grain at tillering as shown in Figure 7. In booting stage, direct relationship was seen between and Zn in rice grain, it may be due to increasing dose of P and Zn fertilizer as shown in Figure 8. The same was confirmed by Amanullah et al. (2020) in their study, they found that increasing P and Zn maximise Zn content in grain, while absence of Zn in applied fertilizer has reduced its content in rice grain (Gianquinto et al., 2000).

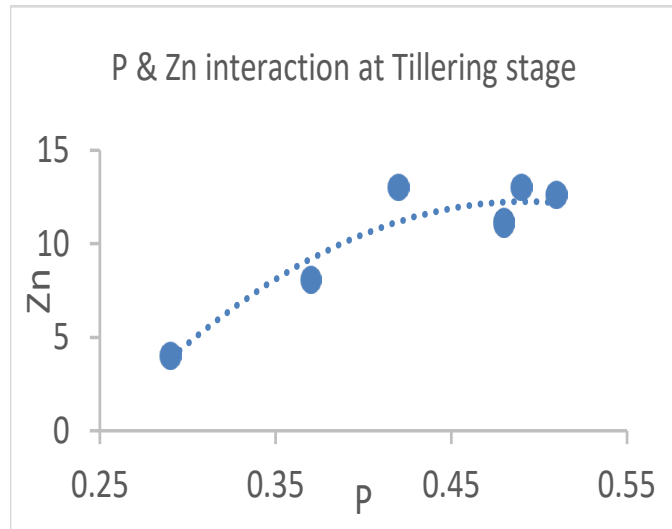


Figure 7: P and Zn interaction at tillering stage.

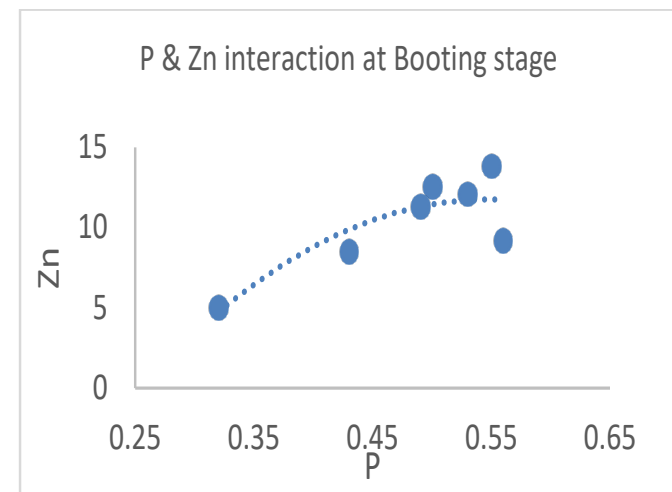


Figure 8: P and Zn interaction at booting stage.

Interaction between phosphorus and phytic acid in rice

P × phytic acid interaction revealed that an increase in P level directly influenced Phytic acid content in grains at both growth stages under study i.e. tillering and booting stage of rice as illustrated in Figures 9 and 10.

Interaction between zinc and phytic acid in rice

Interaction between Zn and phytic acid has shown a clear polynomial trendline which depicts that increase in zinc increases PA content in rice grain as shown in

Figures 11 and 12.

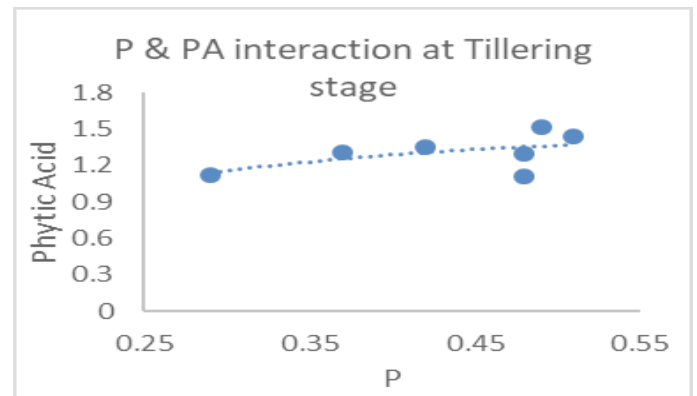


Figure 9: P and PA interaction at tillering stage.

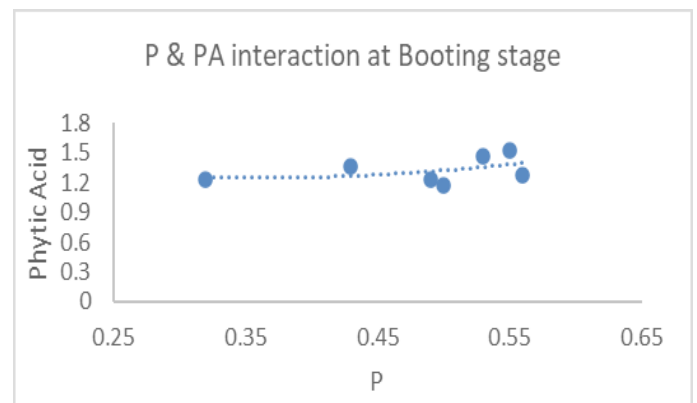


Figure 10: P and PA interaction at booting stage.

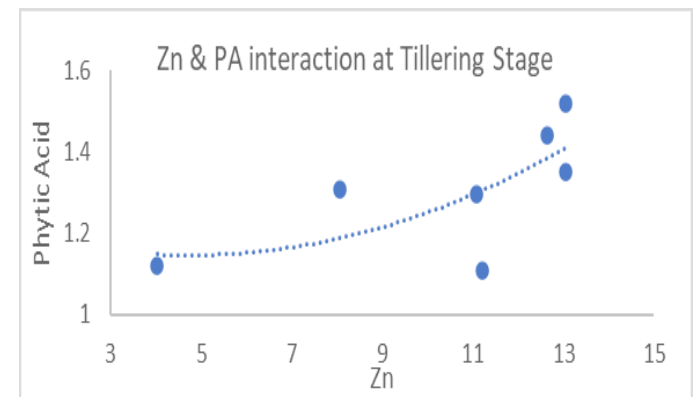


Figure 11: Zn and PA interaction at tillering stage.

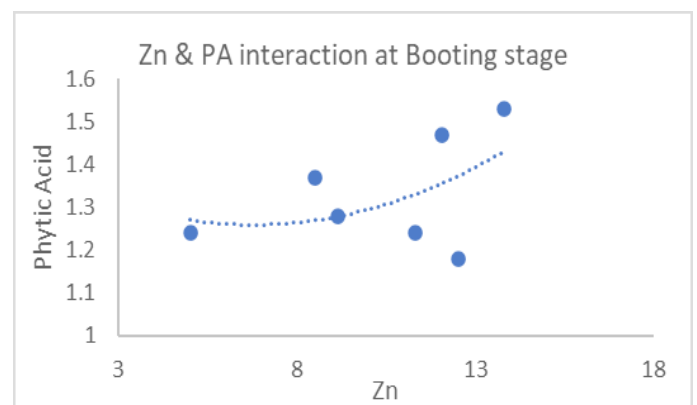


Figure 12: Zn and PA interaction at booting stage.

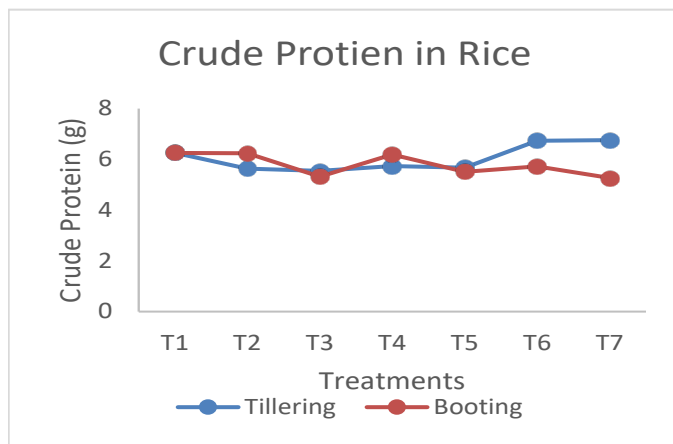


Figure 13: Impact of P and Zn application on rice crude protein (g).

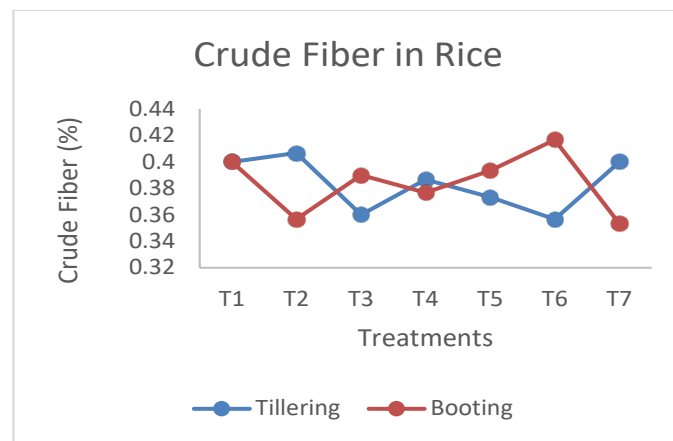


Figure 14: Impact of P and Zn application on rice crude fiber (%).

Quality attributes of rice

Crude protein in rice has shown a random effect by zinc application at both stages i.e., tillering and booting stage of rice, as shown in Figure 13. Maximum crude protein detected in T₆ and T₇ (6.73 and 6.75 g, respectively) when zinc was applied at tillering stage as compared to control where it shows 6.25 g. Random crude fibre in rice has been observed with more strength in response to zinc application in above mentioned stages as shown in Figure 14. Maximum crude fibre has been reported in T₆ at booting stage i.e., 0.42% as compared to 0.40 % of control. Crude fibre concentration is usually low in polished rice (Eggum, 1979). In quality parameters crude protein significantly increased by the Zn application as compared to NPK alone (Sudha and Stalin, 2015).

Conclusions and Recommendations

This study clearly depicts positive interaction of P and Zn on rice growth, yield and nutrient attributes. Zinc applied at panicle initiation stage showed significantly better results in most of the parameters.

Novelty Statement

There is positive interaction between P and Zn when applied to rice.

Author's Contribution

Farah Rasheed: Concept and designing of work, execution of study and manuscript write up.

Ana Aslam: Data interpretation and graphical representation.

Muhammad Aftab and Ghulam Sarwar: Final editing and proof reading

Raheela Naz and Hina Nazir: Statistical analysis of data

Sadia Sultana and Amina Kalsom: Helped in lab. work

Nisa Mukhtar and Ifra Saleem: Elaborated results and discussion

Qudsia Nazir, Adnan Rafique and Muhammad

Arfan-ul-Haq: Worked on materials and methods

Abid Niaz and Muhammad Arif: Participated in introduction portion

Aamer Sattar and Sarfraz Hussain: Overall supervision and guidance about manuscript write up.

Conflict of interest

The authors have declared no conflict of interest.

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