QUALITATIVE AND CHEMICAL ANALYSIS OF RICE KERNEL TO TIME OF APPLICATION OF PHOSPHORUS IN COMBINATION WITH ZINC UNDER ANAEROBIC CONDITIONS

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ABSTRACT

A field experiment was conducted to study the effect of different application timings of P in combination with Zn and P alone on rice at Adaptive Research Farm, Gujranwala, Pakistan during the year 2010. The experimental treatments include: P application at the time of last puddling, P application just before transplanting, P application 10 days after transplanting, P application 20 days after transplanting, P + Zn application at the time of last puddling, P + Zn application just before transplanting, P + Zn application 10 days after transplanting and P + Zn application 20 days after transplanting. Different application timings of P in combination with Zn significantly reduced the sterile percentage, when application delayed which resulted in more normal kernels as compared to application of P alone and late one. Maximum abortive and opaque kernels were observed when P application was done just before transplanting. Maximum chalky kernels were perceived when P+Zn were applied 10 days after transplanting.Regarding concentration of P and Zn in rice straw and grain significant difference was observed in all treatments. The highest Zn concentration in grain and straw was observed in P + Zn application at 20 days after transplanting, while minimum Zn concentration was observed in all treatments where P alone was applied in different timings. P concentration in grain and straw was observed near seedling transplanting time more as compared to late applications of P alone and in combination with Zn. It is concluded that Zn is essential nutrient for anaerobic rice. More ever best timing for P application in rice crop is at time of last puddling. Combine application of Zn and P in rice crop at the time of last puddling, resulted in higher yield.
Keywords: Rice, Time of application, Zn, P, Yield and Qualitative Characteristics

INTRODUCTION

Rice (*Oryza sativa* L.) is the second largest staple food crop after wheat in Pakistan and is also a major source of export earnings in recent years. It adds for 4.4 percent of value added in agriculture and 0.9 percent in GDP. Pakistan grows high quality aromatic rice to meet both local market demand and exports. Area cultivation under rice crop is estimated as 2571 thousand hectares, with the total production of 6160 thousand tons, giving an average yield of 2396 kg ha^{-1} (Economic Survey of Pakistan, 2011-12).

Among other macro nutrients phosphorus (P) is an essential element for vegetative growth and development. It plays basic role in metabolic and energy producing reactions in plants. It is a vital part of nucleic acids and is necessary for cellular respiration and in the metabolism of the starch, protein and fats (Yaseen et al., 1999). Application of P stimulates blooming and seed formation in rice. Proper use of P will help in enhancing yield per unit area in terms of normal kernels (Demkin and Ageev, 1990). It plays major physiological role in the accumulation and release of energy during cellular metabolisms resulting in chalky kernels (Khalil et al., 2002). Furthermore its significance is highlighted when spikelets endure sterile (Jalil et al., 1990; Mythili et al., 2003) and kernels remain abortive (Alam et al., 2009; Okonji et al., 2012).

As long as 70 years ago, Zinc (Zn) was recognized as essential micronutrient (Sommer and Lipman, 1926). Rice has been reported more prone to Zn deficiency than upland crops like wheat rice cropping system (Khan et al., 2007a; Kausar et al., 1976). Condition persist in paddy growing soils are mostly not fit for the availability of Zn element and thus deficiency of Zn has been observed all over the

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country in rice cultivation areas (Bhatti and Rashid, 1985). Zn deficiency is a major micronutrient disorder in alkaline calcareous soils of the Pakistan (Anonymous, 1998) and elsewhere (Takkar and Walker, 1993) because of availability and high Zn fixation. Due to clayey, alkaline and calcareous nature of soils in Pakistan, Zn fertilizer is mainly fixed by soil particles and very low amount is available and uptake by rice crop plants (Tahir et al., 1991). This uptake is reflected in kernels and plants for both Zn as well as P (Devarajan and Ramanathan 1995, Yaseen et al. 1999 and Srivastava et al. I999). Zn deficiency is more common under the conditions of high pH, calcareous, high P levels, light and sandy soils, and wet soils. Qi (1987) reported that the available Zn in the soils correlated negatively with soil pH and $CaCO_3$ content and positive with clay content.

Deficiency of Zn is the most widespread micro-nutrient deficiency in paddy cultivated soils. Zn deficiency is common in lowland conditions due to reducing environment that develop following; submergence, with Zn becoming unavailable after reacting with iron compounds and other meta-stable compounds formed in reducing reactions (Li et al., 2011). Most of the applied P becomes unavailable to growing plants due to alkaline and calcareous nature of the soils of Pakistan (Tahir et al., 1991). The low availability of P is caused by its fixation. The efficient use of phosphatic fertilizers depends on the crop requirements, rate and time of application and placement methods. Zn deficiency is common in calcareous soils where high pH reduces Zn availability. P after its absorption interacts in a complex manner with Zn, Mn, Fe and Cu affecting their mobility and translocation in the plant system which outcomes in chalky and opaque kernels (Naik and Das, 2007).

Among different methods of P and Zn application, fertilizer application after transplanting is the common practice of rice cultivation in Pakistan. Special nutrient management practices (especially for N & P) are required for improving yield. Method, dose and time of application of fertilizers are vital for securing higher yields (Khan, 2002; Oh et al., 1990; Sahoo et al., 1990; Irshad, 1996). However, the question of proper application of P and Zn at proper time remained an unquenched question which agitated the agronomists to look into this matter. Keeping in view the P and Zn role on the yield and performance of rice at different times, a study was planned with the objectives to find the appropriate P application timing in rice under anaerobic condition, and to study the interactive effect of P and zinc application on rice yield at different times of application.

MATERIALS AND METHODS

A field experiment was conducted to study the response of fine rice (*Oryza sativa* L.) to time of application of P in combination with Zn under anaerobic conditions, at Adaptive Research Farm, Gujranwala, Pakistan during Kharif, 2010.

The experiment was laid out in randomized complete block design along with four replications. The net plot size was measured 3 m x 5 m and rice seedlings were planted at hill to hill distance of 22.5 cm using two seedlings per hill. Rice variety "Super Basmati" was used as experimental medium of the trial. Thirty five days old nursery seedlings were transplanted manually in a well puddled field. The experiment was comprised of: T_1: P application at the time of last puddling, T_2: P application just before transplanting, T3: P application 10 days after transplanting, T_4: P application 20 days after transplanting, T_5: P + Zn application at the time of last puddling, T_6: P + Zn application just before transplanting, T_7: P + Zn application 10 days after transplanting, T_8: P + Zn application 20 days after transplanting.

Giving one deep ploughing with disc plough followed by cultivation and planking twice the experimental field was prepared. The waste matter (roots, leaves, etc.) of previous crop was incorporated in the soil. The land was then kept open for drying and killing soil-borne insects and pathogens. After that the field was puddled for transplanting of rice seedling. For conventional soaking treatment (a common practice for preparation rice nursery), seeds were placed between the two layers of saturated jute bags up-to just appearance of radicles for about 48 hours (Basra et al., 2003). Recommended dose of NK (150-60 kg ha^{-1}) fertilizers was applied in the form of Urea and Potassium Sulphate. All potash and half of N were applied before sowing while the second half of N was applied at panicle initiation stage. The crop was irrigated at continuous intervals according to need of crop. Irrigation was stopped about two weeks before harvesting of crop when the signs of physiological maturity appeared. The crop was grown at a weeds free site and weeds which emerged during crop growth and development were manually pulled from whole of the experimental area. All other agronomic operations were kept normal and uniform for all the treatments. Harvesting was done manually when panicles were ripened fully and threshing of each treatment was done separately.After harvesting and threshing, the clean rough rice of each plot was air-dried and weighed to record the grain yield.

Those kernels which were unfilled and unfertilized were separated from each panicle and then average was calculated to find out sterile spikelets by the formula given by Nagato and Chaudhry, (1969). Sterility percentage = Sterile spikelets/ Total No. of spikelets x 100. After that twenty kernels were randomly selected from each plot to calculate opaque kernels (%). After de-husking, the kernels were kept on a glass board having a bulb illuminated underside in board. The number of opaque kernels was counted and then these were converted into percentage. Subsequently, twenty kernels were selected randomly from each plot then de-husking to determine abortive kernels (%). Kernels spread on bulb illuminated glass board then counted number and converted in percentage. Later on, chalky kernels (%) were visually separated from normal kernels on the basis of chalkiness presence in kernels that observed with help of high resolution magnifying lenses. Lastly, normal kernels (%) were calculated those that attain full size, translucent, show normal starch compaction and normally light to pass through them. The clear translucent and without any chalky spots kernels were calculated by deducting all the other abnormal kernels from total number of kernels.

Chemical analysis of plant and grain

After harvesting, grain and straw samples for all the treatments were oven dried at 70C to a constant weight. Samples were ground and stored in plastic bottles for chemical analysis. First of all, plants material was washed with distilled water to clean it from dust and dirt. After air drying the samples were chopped with a stainless steel knife and placed in china dishes for 12 hours in an oven adjusted at 70 ºC. When dried and become brittle, the samples were grinded in a thoroughly cleaned mortar using wooden pestle and stored them in clean jars. The grains from various treatments were air dried and then well grind with mortar and pestle. Then one gram oven dried plant material was digested in 20 ml concentrated HNO_3 and 10 ml of 72% $HClO_4$, cooled the digest, transferred to 100 ml volumetric flask and made volume (Olsen et al., 1954). Phosphate P in acid digest containing selenium could not be determined using the ascorbic acid/molybdate blue color method. Therefore, phosphate was determined by formation of a yellow phospho-vanado-molybdate complex absorbed at 420 nm. Reagents used in process are: (i) 0.25 percent ammonium vandate NH_4VO_4, Solution. It was prepared by dissolving 2.5 g of NH_4VO_4 in 500 ml boiling water, which was somewhat cooled and then 2.0 ml of concentrated HNO3 was added. The solution was cooled and diluted to one liter. (ii) 5 percent ammonium molybdate solution. It was prepared by dissolving 25 g of $(NH_4)_6 MoO_7.4H_2O$ in 400 ml of distilled water, warmed to 50ºC, the solution was cooled, filtered, diluted to 500 ml. mixed and stored in an amber glass bottle. An aliquot of standard or test P solution that contained 0.5 to 1.0 mg P was filtered into 50 ml volumetric flask, 5 ml of 0.25% NH_4VO_4 was added. The solution was mixed with; 5 ml of 5% ammonium molybdate solution was added and thoroughly mixed. After 10 minutes yellow color was developed whose intensity was read spectrophotometrically at 420 nm with spectronic 21. 5 ml of the digested aliquot was taken in 50 ml volumetric flask, added 5 ml of ammonium vanadate (0.25%) and ammonium molybdate (5%), made volume and allowed to stand for 15-30 minutes. Reading was recorded on spectrophotometer. Then from the standard curve, P concentration (%) in plant was calculated.

Plant material was prepared by dry ash method for the determination of Zn content in plant tissue as described by Raimi and BussIer (1975). Reagent used was 0.7 N H_2S0_4solution. One gram of oven dried plant tissue was taken in Silica crucibles. Placed crucible in cold electric furnace and heat it at 470 °C for two hours. Cool the furnace, removed samples and added to it 10 ml 0.7 N H_2SO_4. Swirled slightly and allowed to stand for 1 hour with occasional mixing. Acid-sample mixture was filtered into 50 ml volumetric flask using Whatman No.42 paper. Drained thoroughly and rinsed the crucibles with two 5.0 ml aliquotes of 0.7 N H_2SO_4, filtered into volumetric flask. Drained thoroughly and washed residue on filter with 10.0 ml 0.7 N H_2SO_4, followed by 10 ml deionized water then the volume made up to mark with deionized water. Zn in the solution was determined by atomic absorption spectrophotometer as given in the Zn determination in the soil extract.

The collected data were analyzed statistically using Fisher's analysis of variance technique and treatment means were compared by Least Significant Difference (LSD) test at 5% probability level (Steel et al., 1997). The data were analyzed by the "MSTAT-C" statistical package on a computer (Freed and Scott, 1986).

RESULTS & DISCUSSION

It is clear from the Table 1, that the maximum sterile spikelet percentage (8.20%) was recorded in P + Zn application 10 days after transplanting, followed by P + Zn application at the time of last puddling (7.56 %) and was at par with P application 20 days after transplanting (7.21 %), While minimum sterile spikelets percentage (6.02%) was observed in P application 10 days after transplanting and was at par with P + Zn application just before transplanting (6.15%). It is also evident from the table that application of Zn along with P increased the value of percent filled grains significantly over application of P only which might be due to adequate supply of Zn that increases the availability and uptake of other essential nutrient resulting improved metabolic activities of rice plants. Similar trends were found in the findings of Jalil et al. (1990), Mythili et al. (2003) and Qi (1987).

Data regarding the percentage of abortive kernels are given in Table 1,which indicated that maximum abortive kernel percentage (4.58%) was observed in P application just before transplanting followed by P application 10 days after transplanting (3.78%) and was at par with P + Zn application 20 days after transplanting (3.68), P + Zn application at the time of last puddling (3.42%), P application at the time of last puddling (3.42 %) and P + Zn application just before transplanting (3.35%) . Minimum abortive kernel percentage (2.11%) was observed in P application 20 days after transplanting followed by P + Zn application 10 days after transplanting (3.21%).These results are in line with the findings of Okonji et al. (2012), Alam et al. (2009) that P applications just after transplantation results in minimum up take which might probability hinder the uptake of Zn and consequence in reduction in kernel yield.

Data regarding opaque kernels percentage are given in Table 1. The table showed that maximum percentage of opaque kernels (14.43 %) was observed in P application at the time of last puddling and was at par with P + Zn application 10days after transplanting (13.98 %) while minimum percentage of opaque kernels was found in P application just before transplanting (11.26 %) and was at par with P application 10 days after transplanting (12.20%) and P application 20 days after transplanting (11.68%). Similar trends were reported by Alam et al. (2009), Okonji et al. (2012) and Yash et al. (1992) that maximum absorption is done when P is applied at the time of last puddling, due to which kernels become opaque and show poor nutritional uptake of carbohydrates. The reason might be due to inadequate supply of Zn (Takkar and Walker, 1993).

It is clear from Table 1 that maximum chalky kernels percentage (26.15 %) was observed in P + Zn application 10 days after transplanting which was statistically same with P application 10days after transplanting (25.23 %), P application 20 days after transplanting (24.67%) and P + Zn application just before transplanting (24.65 %), while minimum percentage of chalky kernels was found in P application just before transplanting (21.35 %) and it was at par with P application at the time of last puddling showing the chalky kernel percentage of 21.48 %. These results are in accordance with the findings of Khan et al. (2007b), Malik et al. (2011), and Muthukumararaja et al. (2012) that P+Zn application 10 days after transplanting tends to accumulate carbohydrates in maximum quantity in the kernel.

Data regarding normal kernels percentage are presented in Table 1, which indicated significant effect of different P application timing alone and in combination with Zn on normal kernels percentage. Maximum normal kernel percentage (57.73 %) was recorded in P + Zn application at the time of last puddling and was at par with P application just before transplanting (56.66%). On the other hand minimum normal kernel percentage (50.59%) was observed in P application 10 days after transplanting and which was at par with P + Zn application 10 days after transplanting

(50.64%). These results are in harmony with the findings of Alam et al. (2009), Okonji et al. (2012) and Ahmed et al. (1992) that P in combination with Zn at the time of last puddling is best utilized and its consequences (in terms of kernel yield) are reflected. These results are in accordance with the findings of Yaseen et al. (1999), Li et al. (2011) and Ahmed et al. (1992) that in P + Zn application at the time of last puddling results in maximum normal kernels so that practice should be advocated to be promulgated.

It is clear from the Table 1, that kernel yield was significantly affected by different time of P application in combination with Zn. The highest kernel yield (4.38 t ha^{-1}) was recorded in P + Zn application at the time of last puddling followed by 3.90 t ha^{-1} for P + Zn application just before transplanting. Minimum kernel yield (2.96 t ha^{-1}) was observed in P application 20 days after transplanting which was at par with P + Zn application 20 days after transplanting (3.18 t ha^{-1}) and P application 10 days after transplanting (3.08 t ha^{-1}). Yield decreased as the time of application of P delayed. Similar results were reported by Slaton et al. (2002), Oh et al. (1990) and Malik et al. (2011) that P + Zn application at the time of last puddling resulted in maximum kernel yield.Increase in yield by the addition of Zn along with P application over P alone application might be attributed to qualitative characteristics of kernel like chalkiness, opaqueness and abortiveness, which were affected significantly by the application of Zn as shown in Table 1. Similar results were reported by Naik and Das (2007), Muthukumararaja et al. (2012) who observed that application of Zn to rice significantly affected kernel yield and quality characteristics.

Data regarding Zn concentration in rice plant are presented in Table 1. It is clear from the table that Zn concentration varied significantly by time of P application timing in combination with Zn. Maximum Zn concentration was recorded in P + Zn application at the time of last puddling (31.06 ppm) which was followed by P + Zn application just before transplanting showing the Zn concentration of 28.96 ppm and was at par with P + Zn application 20 days after transplanting (28.35 ppm). On the other hand lower Zn concentration in P application at the time of last puddling was recorded (24.38 ppm) and it was statistically similar with P application 10 days after transplanting (24.68 ppm). Zn along with P fertilizer increased the Zn content in rice plants significantly over application of P. The data showed that application of Zn had increased the Zn contents of straw which might have been owing to more vigor of plants and greater root activity for the uptake of this element. Similar results were reported by Srivastava et al. (1999), Malik et al. (2011) and Yaseen et al. (1999) that Zn application along with P at the time of last puddling magnify the additive role of Zn to multiplicative in quality as well as final kernel yield.

It is evident from the data given in Table 1, that the concentration of P in grains was affected significantly by P application timing in combination with Zn. Maximum P concentration in grain (0.3768 %) was recorded in P application at the time of last puddling followed by P application just before transplanting (0.3415). Minimum P concentration in grains (0.2860) was recorded in P + Zn application 10 days after transplanting which was at par with P + Zn application just before transplanting (0.2880%), P + Zn application at the time of last puddling (0.2895%) and P + Zn application 20 days after transplanting (0.2912%). There was a progressive decrease in P content in grains with application of zinc, which might be due to the antagonistic effect of Zn on P absorption. Chaudhry et al. (1992) and Yaseen et al. (1999) also reported similar results. While highest concentration was of P observed in T_1 (P application at the time of last puddling) and overall trend of P concentration decreased as the time of application was delayed which are contradictory, to those reported by Slaton et al. (2002), Alam et al. (2009) and Baskar et al. (2000) findings who stated that P concentration in harvested grain was not affected by time of P fertilizer application.

It is clear from table 1, that Zn concentration in grain was significantly affected by the time of P application in combination with Zn. The highest Zn concentration 23.25 ppm was recorded in P + Zn application 20 days after transplanting and it was at par with (23.05 ppm) in P + Zn application at the time of last puddling and P + Zn application 10 days after transplanting (23.01 ppm). Minimum Zn concentration (17.00 ppm) was observed in P application at the time of last puddling followed by P application 20 days after transplanting (18.01 ppm). Moreover, the effect of application of P timings (T_1, T_2, T_3 and T_4) was significantly different from Zn along with P (T_5, T_6, $T_7$and T_8) to enhance the availability of soil Zn which might have been due to the improvement of enzymatic function, metabolic processes of the plant and Zn application which ultimately have increased the uptake of Zn. Almost similar results were reported by Devarajan and Ramanathan (1995), Yaseen et al. (1999) and Srivastava et al. (I999) who reported that Zn application to soil increased the total biomass, kernel yield, Zn concentration in the grain and uptake of Zn in plant and the grains.

Perusal of the Table 1 indicated that more concentration of P in rice plant (0.0865%) was recorded in P application just before transplanting and at par with P application at the time of last puddling that have (0.0842%) percent P.

The minimum P concentration (0.0705%) was observed in P + Zn application 10 days after transplanting followed by P + Zn application just before transplanting (0.0753%) and it was at par with P + Zn application 20 days after transplanting (0.0738%). P concentration decreased potentially when application of P delayed from sowing to onward. The results are contradicting to those of Slaton et al. (2002), Alam et al. (2009) and Khan et al. (2007b) who reported that straw P concentration increased as the time of application was delayed.

As regard the combine effect of P + Zn there was progressive decrease in P concentration with application of Zn which might be due to the antagonistic effect of Zn on P uptake. These results are in complementary with those reported by Chaudhry et al. (1992) and Yaseen et al. (1999) who stated that P concentration in grain used to decrease potentially when application of P delayed from sowing to onward and vice versa.

Table 1: Kernel Quality and Zn, P concentration in kernel and rice plant as affected by different time of application of Phosphorus in combination with Zinc

S. No	Treatments	Sterile spikelets (%)	Opaque kernels (%)	Abortive Kernels (%)	Chalky kernels (%)	Normal Kernels (%)	Kernel yield (t/ha)	Zn Conc. In rice plant (ppm)	Phosphorus concentration in grain (ppm)	Zinc concentration in grain (ppm)	Phosphorus concentration in rice plant (ppm)
1	**T_1**: P application at the time of last puddling	6.43 de	14.43 a	3.42 bc	21.48 c	54.11 b	3.51 c	24.38 e	0.3768 a	17.00 f	0.0842 ab
2	**T_2**: P application just before transplanting	6.79 cd	11.26 e	4.58 a	21.35 c	56.66 a	3.35 cd	25.59 d	0.3415 b	20.03 c	0.0865 a
3	**T_3**: P application 10 days after transplanting	6.02 e	12.20 cde	3.78 b	25.23 ab	50.59 d	3.08 de	24.68 de	0.3032 c	19.01 d	0.0823 bc
4	**T_4**: P application 20 days after transplanting	7.21 bc	11.68 de	2.11 d	24.68 b	55.08 b	2.96 e	22.58 f	0.3115 c	18.00 e	0.0808 cd
5	**T_5**: P + Zn application at the time of last puddling	7.5550 b	12.35 cd	3.42 bc	20.08 d	57.73 a	4.38 a	31.06 a	0.2895 d	23.05 a	0.0793 d
6	**T_6**: P + Zn application just before transplanting	6.15 e	13.65 ab	3.35 bc	24.65 b	51.56 cd	3.90 b	28.96 b	0.2880 d	20.94 b	0.0753 e
7	**T_7**: P + Zn application 10 days after transplanting	8.20 a	13.98 a	3.21 c	26.15 a	50.64 d	3.52 c	27.09 c	0.2860 d	23.01 a	0.0705 f
8	**T_8**: P + Zn application 20 days after transplanting	6.45 de	12.79 bc	3.68 bc	24.37 b	51.95 c	3.18 de	28.35 b	0.2912 d	23.25 a	0.0738 e
9	**LSD (5%)**	0.55	0.9960	0.54	0.95	1.18	0.3247	1.05	0.0094	0.8994	0.0023

Any two means not sharing a letter in common differ significantly at $P \leq 0.05$

CONCLUSION

It is concluded that Zn is essential nutrient for anaerobic rice. More ever best timing for P application in rice crop is at time of last puddling. Combine application of Zn and P in rice crop at the time of last puddling, resulted in higher yield.

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