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Effect of Phosphorus in Alleviating Arsenic Toxicity in Paddy (*Oryza sativa* L.) under Hydroponic System

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Abstract

In this experiment, effects of phosphorus (P) in reducing arsenic (As) uptake in paddy were studied under hydroponic system. The result recorded non-significance effect of As over number of leaves of rice. There was a considerable decrease in shoot height, shoot fresh weight and dry weight. Addition of 40 ppm P improved plant height, shoot fresh and dry weight, root length, root fresh weight and root dry weight, significantly. N and K content, were improved with increased P application. Highest shoot P content of 0.38% and shoot K content of 1.75% were recorded in treatment $T_{9.}$ A similar pattern was also found for rice root, where highest P content of 0.49% and K content of 2.86% were recorded in treatment $T_{9.}$ Highest shoot As of 931 µg kg^{-1} and root As of 1.61 mg kg^{-1} were found in the treatment T_1. Addition of 40 ppm P significantly lowered the shoot As content to 548.67 µg kg^{-1} which was 69.68% decrease and 2.09 times decrease in root As was found as compared to control (treatment T_1). Finally, external application of 40 ppm P has ameliorating effect over As toxicity and significantly reduce As content in rice.

Keywords: Arsenic, Hydroponic, Paddy, Phosphorus, Toxicity

Introduction

Arsenic is known as toxic element, long time exposure to it causes cancer, now-a-days it has become a global concern (Bundschuh *et al.*, 2022). Many people believe that arsenic is not necessary for plants and other living things and its toxicity occurs when the amount exceeds the threshold limit (Mondal, 2023). It has adverse effects on the health of animals and plants and humans (Mondal, 2023). In plants, As toxicity cause severe harmful effect on morphological and physiological activities and reduces the crop yield drastically (Matsumoto *et al.*, 2016). When paddy is grown under submerged condition anaerobic environment develops which makes As more mobile, toxic and easier for paddy to absorb (Suriyagoda *et al.*, 2018). Rice is considered as staple food in South Asian nations like India, China, Indonesia and Bangladesh and generally cultivated under submerged puddled conditions (Reddy *et al.*, 2023). Appreciable accumulation of As by paddy is generally found under continuous submerged and anaerobic soil condition (Shahid *et al.*, 2018). Soil redox potential (Eh), pH, organic matter, phosphate, sulfate, manganese and iron regulate As speciation and its availability (Upadhyay *et al.*, 2019). The overall amount of arsenic in the soil typically varies between 0.1 and 10 mg kg^{-1} in uncontaminated soil (Zhao *et al.*, 2010). Safe limit of total soil As for crop cultivation should be < 20 mg kg^{-1} (Kumarathilaka *et al.*, 2019). The World Health Organisation (WHO) recommends < 0.2 mg kg^{-1} as safe limit of As in polished rice grain (Biswas *et al.*, 2020). Chandigarh was the first place in India where groundwater contamination from As was discovered (Datta and Kaul, 1976). Recently, it was discovered that the groundwater in the Varanasi area of Uttar Pradesh contains As (Chattopadhyay *et al.*, 2020). When crops are irrigated with As contaminated groundwater, As naturally builds up in the plants (Chou *et al.*, 2016). Arsenic uptake also influenced by crop species, rooting patterns, agronomic management techniques and soil type (Majumder *et al.*, 2021). A recent research by Samal *et al.* (2021) found that high yielding rice cultivars adsorbed more As than indigenous types. In rice, As accumulation is highest in roots compare to the uppermost portion (Liu

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et al., 2004). In the upper part of rice plant, straw had a greater concentration of arsenic than grain and husk portions (Bhattacharya *et al.*, 2010). Various agronomic management strategies including aerobic cultivation, alternate wet-dry, intermittent flooding, use of hyperaccumulating crops, high quantities of phosphorus, iron and silicon fertilisers are more efficient in lowering the availability and uptake of As in paddy (Suriyagoda *et al.*, 2018). Phosphorus fertilization is the common agriculture method for improving yield of rice and reported to suppress As uptake (Islam *et al.*, 2016). But still, the actual level of phosphorus to prevent As uptake for paddy locally grown under Varanasi region of Uttar Pradesh is unknown. Interaction of As and P in soil is very critical and sometimes results are contradictory, thus study under a hydroponic system is the best alternative which removes the effect of soil (Anawar *et al.*, 2018). Consequently, our objective of study was to identify the critical phosphorus concentration for averting As uptake by paddy under a hydroponic system.

Material and Methods

Pot Experiment Design

A hydroponic system was used for the experiment in the year of 2018-2019 using paddy (HUR-105) as a test crop, in the net-house of the department at BHU, Varanasi (Latitude: 25.2652° N and Longitude: 82.9910° E), India. Plastic pots are filled with 5 kg of perlite then Hoagland solution was added as the source of nutrition and finally As and P were added as per the treatment combination. Perlite is an inactive substance and only acted as a root anchorage for plants growth and support under the hydroponic system. Perlite was purchased from Nirmal Interlinks, Lucknow, UP. Three numbers healthy paddy saplings (15 days old) were then transplanted to each pot and irrigated with Hoagland solution. Treatment comprises with increasing doses of phosphorus of 0, 5, 10, 15, 20, 25, 30, 35 and 40 ppm with arsenic of 10 ppm then applied to the pots. All the plants were maintained for next 15 days. Finally, root length, root fresh weight and dry weight, number of leaves, shoot height, shoot fresh weight and dry weight, were measured.

Processing of Plant Samples

Plant samples were dried in a hot air oven at 65±2 °C for 8 hours per day to achieve static weight. A Wiley stainless steel mill grinder was used to ground the samples, then sieved (20 mesh) and stored (Tandon, 1993).

Plant Samples Digestion for Estimation of Nitrogen

In a digestion glass tube 0.5 g plant sample was taken and dissolved with 10 mL of acid solution that comprised of H_2SO_4 and $HClO_4$ (9:1 v/v) and left for overnight. Then, 10 g of sulphate mixture and catalyst were added. Finally, in a digestion chamber the mixture was heated to obtain colourless solution. For final estimation, distillation was done under a micro-Kjeldahl distillation instrument followed by boric acid titration (Tandon, 1993).

Plant Samples Digestion for Estimation of Phosphorus

One gram (1 g) plant sample was placed in a glass digestion tube then 10 mL tri-acid mixture (HNO_3 : $HClO_4$: H_2SO_4 in the ratio of 9:3:1) was applied, on the next day digestion was done to obtain colourless solution. Then, vanadate molybdate solution was added which developed yellow colour, finally, concentration of phosphorus was measured under a spectrophotometer at 440 nm wavelength by using a blue filter (Tandon, 1993).

Estimation of Potassium

Digested extract after dilution and filtration was used for potassium estimation using a Flame Photometer (Tandon, 1993).

Digestion and Estimation for Arsenic

For digestion of plant samples method of Meharg and Jardine (2003) was followed. All As species reduce to As(III) by addition of concentration HCl, 5% KI and 5% ascorbic acid. For further reduction of As(III) to AsH_3 (gas), 0.5% $NaBH_4$ and 0.5% NaOH solution were added. Reading was recorded at 193.7 nm wavelength of cathode lamp under a Vapour Generation Atomic Absorption Spectrometry (Agilent Technologies, Serial no. MY16020008).

Statistical Analysis

Statistical analysis was done under completely randomized design (CRD) with nine treatments and three replications. Significance level was set at P≤0.05. SPSS 16.0 software was used for the calculation.

Results and Discussion

Effect of Arsenic and Different Doses of Phosphorus on Number of Leaves, Shoot Length, Shoot Fresh Weight, Shoot Dry Weight of Paddy

The highest shoot length was 32 cm, recorded in T_9 treatment, whereas the minimum shoot length was 17.83 cm where treatment T_1 was applied (Table 1). Application of phosphorus and arsenic did not affect the total number of leaves of rice and their effects were non-significant (Table 1). With higher P dosages, a significant change in shoot length was recorded between T_1 and T_9 (Table 1). Shoot length increased by 79.42% in T_9 compared to T_1. T_9 had the highest shoot fresh weight (5.27 g), whereas T_1 had the lowest (3.88 g). It was revealed that shoot fresh weight was increased significantly with increased doses of P from 0 to 40 ppm (Table 1). Shoot dry weight also increased significantly from T_1 to T_9. Compared to no P addition, the treatment that received 40 ppm of P showed 56.41% increase in shoot dry weight. T_9 had the highest shoot dry weight (0.61 g), while T_1 had the lowest (0.39 g) (Table 1). The detrimental effects of As were gradually lessened with an increase in P dosage and as a result, the number of leaves, shoot length and shoot fresh weight and dry weight all improved.

Effect of Arsenic and Different Doses of Phosphorus on Root Length, Root Fresh Weight, Root Dry Weight of Paddy

Root length of rice increased significantly in the treatment T_9 which received P 40 ppm compared to no application of P (Table 2). Root length of rice was recorded 7 cm in T_1 (P dose 0 ppm) but it was increased to 13.73 cm which is about 96.14% increase at T_9 when the dose of P was 40 ppm in (Table 2).

Table 1: Effect of arsenic and different doses of phosphorus on number of leaves, shoot length, shoot fresh weight, shoot dry weight of paddy grown in Hoagland solution medium

Treatment	Notation	Number of leaves	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
T_1	$As_{10}P_0$	2.33±0.33	17.83±1.48	3.88±0.12	0.39±0.02
T_2	$As_{10}P_5$	2.33±0.33	21.77±1.01	4.35±0.10	0.47±0.02
T_3	$As_{10}P_{10}$	2.67±0.33	22.40±1.45	4.43±0.14	0.49±0.01
T_4	$As_{10}P_{15}$	2.67±0.33	23.17±1.48	4.57±0.12	0.52±0.02
T_5	$As_{10}P_{20}$	3.00±0.01	24.45±1.31	4.77±0.12	0.52±0.02
T_6	$As_{10}P_{25}$	3.00±0.01	25.67±1.20	4.63±0.11	0.54±0.02
T_7	$As_{10}P_{30}$	3.00±0.01	26.33±1.45	4.92±0.10	0.58±0.01
T_8	$As_{10}P_{35}$	3.33±0.33	30.33±1.20	5.00±0.10	0.60±0.01
T_9	$As_{10}P_{40}$	3.33±0.33	32.00±1.15	5.27±0.13	0.61±0.02
	CD (0.05)	0.81	3.91	0.35	0.05
	S/NS	NS	S	S	S

Table 2: Effect of arsenic and different doses of phosphorus on root length, root fresh weight, root dry weight of paddy grown in Hoagland solution medium

Treatment	Notation	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
T_1	$As_{10}P_0$	7.00±0.58	1.82±0.02	0.27±0.01
T_2	$As_{10}P_5$	11.03±0.55	1.88±0.02	0.29±0.01
T_3	$As_{10}P_{10}$	11.17±0.60	1.91±0.02	0.29±0.01
T_4	$As_{10}P_{15}$	11.77±0.41	1.92±0.02	0.29±0.01
T_5	$As_{10}P_{20}$	12.08±0.46	1.94±0.01	0.29±0.01
T_6	$As_{10}P_{25}$	12.32±0.37	1.98±0.02	0.30±0.01
T_7	$As_{10}P_{30}$	12.97±0.61	1.98±0.01	0.30±0.01
T_8	$As_{10}P_{35}$	13.42±0.68	2.02±0.03	0.30±0.01
T_9	$As_{10}P_{40}$	13.73±0.39	2.05±0.03	0.31±0.01
	CD (0.05)	1.57	0.06	0.01
	S/NS	S	S	S

Significant response was also observed for root fresh and dry weight. Root fresh weight was 12.63% more in T_9 than T_1 (Table 2). Highest root fresh weight (2.05 g) and dry weight (0.31 g) both were also noticed for T_9 and lowest in T_1 (Table 2). T_1 showed a 14.81% decrease in dry weight compared to T_9. Root length was significantly reduced (96.14%) when only 10 ppm As was applied. Our study showed that 40 mg kg^{-1} P was adequate to suppress As uptake by paddy.

Nitrogen, Phosphorus, Potassium and Arsenic Content in Shoot of Paddy Grown in Hoagland Medium along with Perlite used as Anchorage Material

The maximum N content in paddy shoot of 0.82% was found in T_7, T_8 and T_9 treatments and the minimum of 0.72% N was found in T_1. When P was applied at a rate of 25 ppm or higher, a significant change was observed as compared to not applying P. Shoot N content was more as P dosage increased (Table 3). The non-significant increased in potassium (K) content of 1.75% was found in T_9 and lowest of 1.60% was found in T_1 (Table 3). As the P application increases from 0 to 40 ppm, the P content of paddy shoots rises (Table 3). Maximum P of 0.38% was noted in treatment T_9 (40 ppm P), whereas; a minimum P of 0.21% was found in T_1. Shoot P content was 80.95% more in T_9 as compared to T_1 (Table 3). In terms of As concentration of paddy shoot, highest level of As content of 931 µg kg^{-1} was observed in T_1 which received 10 ppm of As and no P and the lowest As content of 549 µg kg^{-1} was found in T_9 which received both 10 ppm As and 40 ppm of P. Highest reduction of As content of 69.68% in treatment T_9 was found due to addition of 40 ppm P (Table 3). With increased doses of P the shoot As significantly reduced (Figure 1).

Nitrogen, Phosphorus, Potassium and Arsenic Content in Root of Paddy Grown in Hoagland Medium along with Perlite used as Anchorage Material

Between T_9 and T_1, there was no significant change in the N% in root (Table 4). However, when the P application rate increased, the P and K content in the root increased significantly. Highest P content of 0.49% was observed in T_9 as compared to 0.11% in T_1. It was observed that T_9 had 4.45 times more P content in root than in T_1. Changes in

Table 3: Nitrogen, phosphorus, potassium and arsenic content in shoot of paddy grown in Hoagland solution medium

Treatment	Notation	Shoot Nitrogen content (%)	Shoot Phosphorus content (%)	Shoot Potassium content (%)	Shoot Arsenic content (µg kg^{-1})
T_1	$As_{10}P_0$	0.72±0.02	0.21±0.01	1.60±0.06	931.00±11.85
T_2	$As_{10}P_5$	0.75±0.03	0.22±0.02	1.63±0.02	789.67±14.68
T_3	$As_{10}P_{10}$	0.75±0.03	0.23±0.01	1.69±0.09	772.00±15.28
T_4	$As_{10}P_{15}$	0.77±0.02	0.29±0.01	1.71±0.05	761.67±14.19
T_5	$As_{10}P_{20}$	0.79±0.02	0.30±0.02	1.71±0.03	745.00±11.36
T_6	$As_{10}P_{25}$	0.81±0.03	0.33±0.02	1.73±0.09	717.33±14.44
T_7	$As_{10}P_{30}$	0.82±0.03	0.33±0.01	1.74±0.13	617.67±14.19
T_8	$As_{10}P_{35}$	0.82±0.02	0.36±0.01	1.74±0.03	595.67±13.86
T_9	$As_{10}P_{40}$	0.82±0.03	0.38±0.02	1.75±0.02	548.67±14.89
	CD (0.05)	0.07	0.04	0.20	41.35
	S/NS	NS	S	NS	S

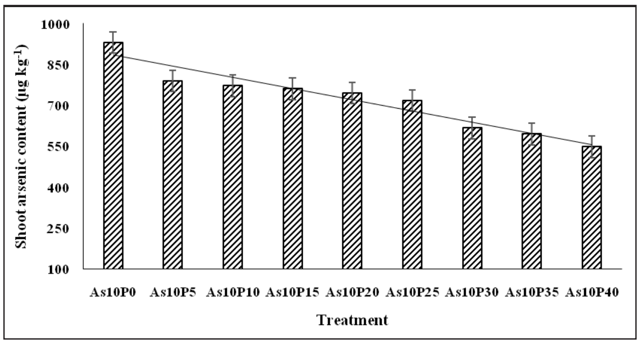

Figure 1: Arsenic content of shoot of paddy grown in Hoagland solution medium

root K content were significant with increased doses of P application. Phosphorous dose of 40 ppm in T_9 had resulted in highest root K content (2.86%). Root K of 23.27% more in T_9 was observed than T_1 (Table 4). Significant reduction in As concentration in root was noticed with increased doses of P (Table 4). T_1 showed maximum amount of As content (1.61 mg kg^{-1}), which gradually decreased to lowest value in T_9 (0.77 mg kg^{-1}). Root As concentration in T_9 was 2.09 times lower compared to T_1 (Table 4). With increased application of P the root As content significantly reduced (Figure 2).

The experiment was carried out under a hydroponic system using perlite as root anchorage material and the Hoagland solution was the only source of all nutrients. Interaction of As and P in soil is very critical and contradictory. Under soil-plant system, As and P can compete for soil adsorption sites and for the uptake by paddy (Lee *et al.*, 2016). Hydroponic system

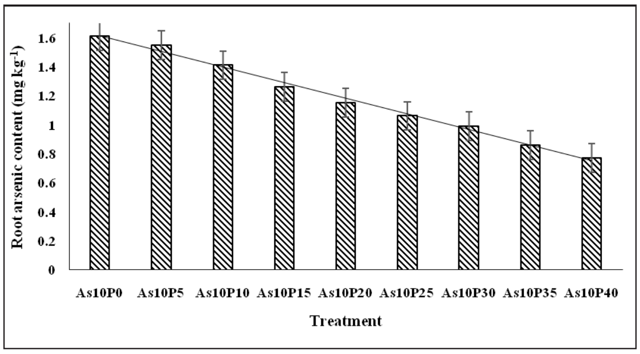

Figure 2: Arsenic content of root of paddy grown in Hoagland solution medium

Table 4: Nitrogen, phosphorus, potassium and arsenic content in root of paddy grown in Hoagland solution medium

Treatment	Notation	Root Nitrogen content (%)	Root Phosphorus content (%)	Root Potassium content (%)	Root Arsenic content (mg kg^{-1})
T_1	$As_{10}P_0$	1.17±0.03	0.11±0.01	2.32±0.17	1.61±0.02
T_2	$As_{10}P_5$	1.16±0.02	0.28±0.02	2.42±0.15	1.55±0.02
T_3	$As_{10}P_{10}$	1.19±0.02	0.32±0.01	2.52±0.12	1.41±0.05
T_4	$As_{10}P_{15}$	1.19±0.01	0.36±0.01	2.74±0.13	1.26±0.05
T_5	$As_{10}P_{20}$	1.19±0.01	0.39±0.01	2.78±0.11	1.15±0.04
T_6	$As_{10}P_{25}$	1.21±0.01	0.42±0.01	2.79±0.12	1.06±0.07
T_7	$As_{10}P_{30}$	1.21±0.02	0.45±0.02	2.82±0.12	0.99±0.01
T_8	$As_{10}P_{35}$	1.21±0.01	0.47±0.01	2.85±0.10	0.86±0.01
T_9	$As_{10}P_{40}$	1.21±0.02	0.49±0.02	2.86±0.10	0.77±0.01
	CD (0.05)	0.05	0.04	0.38	0.11
	S/NS	NS	S	S	S

is the best alternative that eliminates the impact of soil. On the other hand, perlite is a chargeless; it neither adsorbs nor desorbs any elements, making it an inactive substance that adds no nutrition to the solution. Thus, the experimental conditions were solely focussed to find the mutual abilities of As and P in solution medium. Result showed that P can counteract the negative effects of As. Higher concentration of As is phytotoxic to plants and cause reduction in plant growth, yellowing of leaves, stunted roots, reductions in chlorophyll and protein contents and finally reduced crop yield (Islam *et al.*, 2016). They similarly found that sufficient level of phosphate reduced arsenate toxicity and improved plant growth of paddy grown under a hydroponic system (Islam *et al.*, 2016). Because As(V) and P are sister molecules, their transport pathways in rice roots are similar (Cao *et al.*, 2019). At low P concentrations, the transporter gene over-expressed itself, resulting in a significant amount of As uptake, but at higher P concentrations, the transporter gene is likely down-regulated, resulting in less As uptake (Cao *et al.*, 2019). Meharg *et al.* (2009) discovered that 0.5 mM phosphate addition decreased plant arsenate absorption in rice by 75%. Molar ration of P to As of at least 12 is needed to defend crops in contrast to arsenate toxicity (Islam *et al.*, 2016). Even P is preferentially taken up compared to As by the root ion transporters in paddy (Meharg and Macnair, 1994). Our study showed that higher levels of P in solution phase were shown to be more effective in reducing As absorption inside the plant system of paddy. It was also discovered that both fresh and dry weights increased due to enhancement of shoot growth. Hossain *et al.* (2009) described that growth of paddy root, shoot and grain yield were reduced due to As toxicity. Whereas, Howladar *et al.* (2019) concluded that addition of phosphorus reduced As toxicity and improved growth and metabolism of paddy. Additionally, our findings show that external application of 40 ppm P may eliminate toxic effect of As and reduce its content in shoot by 70% of rice plants and improve plant growth. It was discovered in a hydroponic experiment that by using 0.5-1.0 mg L^{-1} of As (V) with higher P doses, the P/As (V) molar ratio increased from 10 to 100 and thereby As toxicity was reduced to a great extent (Huang *et al.*, 2010). In our research experiment same pattern was noticed which clearly explained the reason for reduction in As content in root by several times with application of 40 ppm P. In another experiment, it was observed that with an increment in P level from 0-30 mg kg^{-1}, shoot and root dry weight of paddy were increased and other major micronutrients like P and K also showed the same trend (Howladar *et al.*, 2019). Our research also corroborated with the same thing. This might be due to increase in root iron plague formation, root and shoot growth and competitive effect that resulted in dilution and reduction in As concentration at various parts of paddy (Hu *et al.*, 2015). Several studies described the vital role of P for root growth and expansion and significantly suppresses the harmful effect of As (Anawar *et al.*, 2018). The finding was similar with that of Hossain *et al.* (2009), who stated that the rice grain yield, root growth and shoot growth were all negatively impacted by arsenic. Our study stated that at, lower doses of P, As demonstrated greater competitive ability and significantly reduced P uptake. Consequently, there was greater root anatomical damage, as a result plant nutrient content was also low. But higher doses of P resulted in vigorous root development, which increased the absorption of N, P and K and showed the capacity to reduce the detrimental effects of As.

Conclusion

Thus, it can be concluded that addition of P in solution culture improves shoot height, shoot fresh weight and dry weight, root length, root fresh weight and dry weight of rice variety HUR-105 significantly. N and K content were improved due to increased P application. Higher dose of P treatment reduces As content of paddy shoot by 1.7 times and in roots by 2.09 times as compared to no P application. These results indicate that external application of P has an ameliorating effect on As toxicity by lowering As content in rice. This study stated the important role of P application in minimizing the adverse effects due to the As toxicity in rice. Finally, our study confirms that P treatment has a significant role in lessening the harmful impacts of As in paddy.

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