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Wheat-*Pteris vittata* inter-planting on arsenic contaminated soil to trap arsenic entry into wheat plant

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ABSTRACT

A pot experiment was conducted to reduce the entrance of arsenic into wheat plant by using *P. vittata* as arsenic trapping inter-crop plant with wheat. Experiment was consisted of two densities of the trap plants (*P. vittata*) and control, viz. P_0: No (control); P_1: four plants per m^2 and P_2: eight plants per m^2. Inter-planting of four *P. vittata* per m^2 reduced 97.16% and eight *P. vittata* per m^2 reduced 97.44% arsenic from soil, which could be accumulated into wheat. Maximum yield was found from P_1 (5.7 g per plant) which was statistically similar with P_2 (5.4 g per plant) while minimum was found from P_0 (3.4 g per plant). Highest amount of arsenic accumulation was found from P_1 in wheat grain (1.34 ppm) and straw (35.27 ppm). Arsenic accumulation was found in wheat grain (0.02 and 0.01 ppm in P_1 and P_2 respectively), straw (1.17 and 1.07 ppm in P_1 and P_2 respectively) and. Inter-planting of *P. vittata* in arsenic contaminated wheat field can diminish the arsenic entry into wheat. Regarding both yields of wheat and arsenic concentrations in wheat plant, it can be recommended to inter-plant four *P. vittata* plants per m^2 area as a trap plant.

Key words: *Triticum aestivum*, Chinese brake fern *(P. vittata)*, arsenic reduction and inter-planting

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I. Introduction

Arsenic accumulation in food crops is a major concern. Among the cereals, wheat is second important cereal crop next to rice in economic and consumption aspects in Bangladesh. Most of the agricultural soil is contaminated by arsenic due to the irrigation (Ali *et al.*, 2003; Dittmar *et al.*, 2010) during winter season mostly in southwest part of Bangladesh (Williams *et al.*, 2006; Miah *et al.*, 2005; Islam *et al.*, 2005; Meharg and Rahman, 2003) while half of the wheat is grown on irrigated land during dry season with groundwater. Arsenic contaminated irrigation water and soil can be harmful to wheat seedling at early developmental stages and physiological activities of wheat seedlings are also changed under arsenic stress (Li *et al.*, 2007). Arsenic concentration followed the order: root > shoot > grain in wheat varieties (Karimi *et al.*, 2014). Dietary exposure by human and animal of arsenic contaminated

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wheat grain and also dietary exposure of arsenic contaminated straw by cattle can cause harmful effect on human health directly or indirectly (Abedin *et al.*, 2002a and 2002b; Jahiruddin *et al.*, 2005; Panaullah *et al.*, 2005; Maniee *et al.*, 2009, Geravandi *et al.*, 2011, Ahmadi *et al.*, 2012). While there has been research into the uptake of this metalloid by wheat in total grain, root and shoot levels (Tao *et al.*, 2006; Zhao *et al.*, 2007). But it is important to stop arsenic accumulation into grain as well as straw of wheat plant. *P. vittata* (Chinese brake fern) can survive highly arsenic contaminated soil (4000 ppm) and accumulate up to 27829.7 ppm arsenic (Mayda *et al.*, 2014) and accumulate 23837.2 ppm arsenic from 2000 ppm arsenic contaminated soil (Jamal Uddin *et al.*, 2015) into the plant body. Hyper accumulation is a constitutive trait of *P. vittata* (Zhao *et al.*, 2002). As *P. vittata* can accumulate considerable amount of arsenic from contaminated soil thus it would be potential to reduce the arsenic accumulation into the wheat by using *P. vittata* as trap plant. On the hand, *P. vittata* does not require many nutrients to grow. Interplant *P. vittata* as a trap plant reduced arsenic entrance into rice plant from soil (Mayda *et al.*, 2015). Thus, growing *P. vittata* along with wheat may not interfere (and or less interferences) with the growth and yield. Considering these points in view, the study was undertaken to reduce the arsenic accumulation into wheat plant using *P. vittata* as trap plant.

II. Materials and Methods

Experimental site and period: Experiment was conducted at Department of Horticulture of the Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh and at the Department of Botany of the Jahangirnagar University, Savar, Dhaka-1342, Bangladesh from October 2013 to April 2014.

Treatments and design: Arsenic was applied in the form of Arsenic trioxide (As_2O_3) @ 50 ppm at 7 days before transplanting of the wheat plant. *P. vittata* was inter-planted with wheat into pot as trap plant to mitigate (and/or reduce) arsenic accumulation into wheat plant. Experiment was consisted two densities of the trap plants and a control, viz. P_0: No *P. vittata* (Control); P_1: four *P. vittata* plants per m^2 and P_2: eight *P. vittata* plants per m^2 following completely randomized design with three replication. Wheat seeds were sown broadcasting method where 12-14 plants were grown per m^2 while trap plants were planted at the time of wheat seeds sowing. The pot size was 1.5 m in length, 0.5 m in width and 0.5 m in depth.

Data collection: Data were collected on plant height, number of tillers per plant, number of effective tillers per plant, Number of spikelets, ear length, total number of grains per spike, number of filled grains per spike, number of unfilled grains per spike, percentage of unfilled grains, 1000-grains weight, yield per plant, arsenic accumulation by wheat grain and straw, arsenic accumulation by *P. vittata* and reduction of arsenic accumulation in wheat by *P. vittata* over control.

Chemical analysis for arsenic: Plant biomass was collected and dried then weighted by using precision balance. After that samples were smashed by mortar and pastel machine. The arsenic analysis for was performed by using Atomic Absorption Spectrometer, where use of argon for carrier gas and arsenic was melted by 925ºC; which was approved by ISO organization in Bangladesh Council of Scientific Research Institute (BCSIR), Dhaka, Bangladesh.

50 and 1000 times dilution: (a) 5 ml concentrated HCl was taken at 50 ml volumetric flasks for transferring arsenic into arsenic trioxide and a little bit of distilled water was added. Then 1ml solution was taken very carefully from each volumetric flask to avoid bubble and KI (1 gm) wash added in solution with 150 ml distilled water. After that 0.5 gm sample was taken in volumetric flask and mixed distilled water up to 50 ml and solution turned into yellow color. Another volumetric flask made blank solution, where contain only HCl, KI and distilled water for arsenic analysis. (b) 2 ml solution was taken into 500 volumetric flasks, mixed with distilled water up to 500 ml and shaken very carefully. Then, 05 ml solution was taken into 250 ml volumetric flask and mixed with distilled water up to 250 ml. Again, 5 ml solution from 250 ml solution was taken into 25 ml volumetric flask then 2.5 ml HCl and 2.5 ml KI was added and mixed distilled water, shaking was done very smoothly until turn it into yellow color. Standard solution arsenic was added with HCl 2, 5, 10, 15, 20 ppb respectively.

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Statistical analysis: Data were statistically analyzed using MSTAT-C computer package programme. Difference between treatments was assessed by Least Significance Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

III. Result and Discussion

Plant height

Significant variation was found among the treatments in terms of plant height. Tallest plant was obtained from P_0 (92.0 cm) and shortest from P_2 (82.0 cm) at harvest (Figure 01). Maximum plant height (100.0 cm) harvested in control soil and lowest plant height (87.3 cm) was recorded in 40 ppm arsenic in soil (Asaduzzman *et al*., 2010).

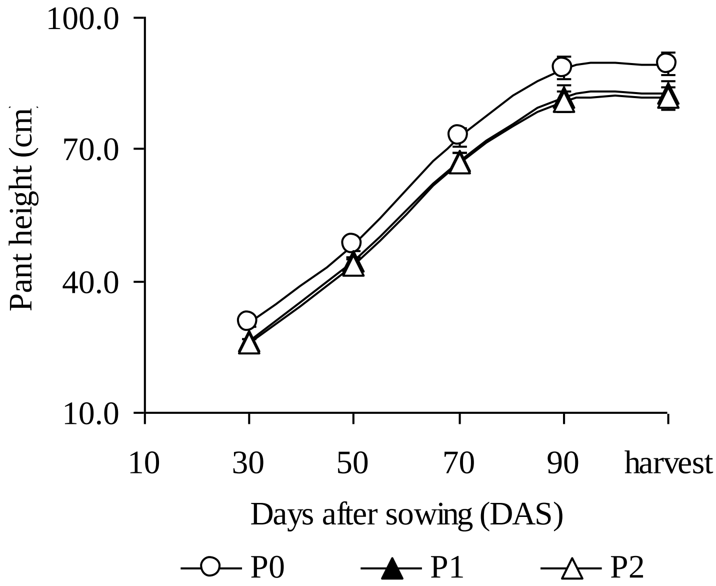

Figure 01. Effect of *Pteris vittata* density on plant height of wheat plant at different days after transplanting (DAT), here, P_0 = Without *Pteris vittata*, P_1 = Four *Pteris vittata* per square meter and P_2 = Eight *Pteris vittata* per square meter

Number of tiller, effective tiller and spikelets

Number of tiller, effective tiller and spikelets at harvest were varied significantly among the treatments. Maximum number of tiller was found from P_1 and P_2 (5.0 per hill) whereas minimum from P_0 (4.7 per hill) at harvests (Table 01). Maximum tiller per hill (6 cm) in control soil and lowest tiller per hill (4.33 cm) was recorded in arsenic contain soil (Asaduzzman *et al*., 2010). Maximum number of effective tiller was found from P_2 (4.7 per hill) whereas minimum from P_0 (3.3 per hill) (Table 01). At low soil arsenic concentration, displacement of soil phosphate by arsenate increased the availability of phosphate to the plant, which results in the increase of plant growth (Duel and Swoboda, 1972; Jacobs *et al*., 1970). Maximum number of spikelets was found from P_0 (18.9 per spike) whereas minimum from P_2 (18.1 per spike) (Table 01). 50 mg per kg arsenic contain soil showed highest number of spikelets (22.6 per spike) and control soil showed 22.3 spikelets per spike in Gaoyou Chinese wheat variety (Zhang *et al*., 2009).

Ear length and total grains

Ear length and total grains were varied significantly among the treatments. Longest ear was found from P_0 (17.0 cm) whereas shortest from P_2 (15.7 cm) which was statistically identical with P_1 (15.8 cm) (Table 01). Inter-planting of *Pteris vittata* on arsenic contaminated soil reduces ear length. *Pteris vittata* uptake arsenic from soil (Ma *et al*., 2001) and can accumulate extremely large concentrations of arsenic in its above ground biomass. For that reasons soil of the control treatments have arsenic thus may responsible for increasing the ear length of wheat. Maximum number of grains was found from P_1

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(30.8 per spike) and minimum from P_0 (29.2 per spike) (Table 01). Maximum grain (47.0 per spike) in control soil and minimum was recorded in arsenic contain soil (36.3 per spike) (Asaduzzman *et al.*, 2010). Arsenic reduces the grain number but when *Pteris vittata* reduce arsenic from soil then wheat produce highest grain in their spike.

Table 01. Wheat responses to *Pteris vittata* density at different attributes

Treatments	Number of tiller/hill	Number of effective tillers/hill	Number of spikelets/spike	Ear length (cm)	Total grains/spike
P_0	4.7 b	3.3 b	18.9 a	17.0 a	29.2 c
P_1	5.0 a	4.3 a	18.5 b	15.8 b	30.8 a
P_2	5.0 a	4.7 a	18.1 c	15.7 b	30.3 b
LSD 0.05	*0.2*	*0.8*	*0.3*	*0.4*	*0.5*
CV %	*6.8*	*8.1*	*0.7*	*1.1*	*0.7*

X P_0= Control (without *Pteris vittata*), P_1 = 4 *Pteris vittata* per square meter, P_2 = 8 *Pteris vittata* per square meter, Y In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

Filled and unfilled grains

Filled and unfilled grains were varied significantly among the treatments. Maximum number of filled grains was found from P_1 (29.2 per spike) which was statistically identical with P_2 (28.5 per spike) whereas minimum was found from P_0 (23.5 per spike) (Table 02). *Pteris vittata* reduce the arsenic soil and enhance the plant growth as a result increase the total number of filled grain. Arsenic contaminated soil adversely affects the filled grain number. Soil arsenic contamination becomes a potential agricultural and environmental hazard worldwide and has been a serious problem for safe food production (Zhang *et al.*, 2009). Maximum number of unfilled grains was found from P_0 (5.7 per spike) whereas minimum was found from P_1 (1.6 per spike) which was statistically identical with P_2 (1.7 per spike) (Table 02). Arsenic inhibited the expression of CAT (Catalase) isozymes but induced the expression of SOD (Super Oxide Dimutase) isozymes at concentrations higher than 5 mg/kg; which indicated arsenic could exert harmfulness in the early development stage of wheat at inappropriate concentrations (Li *et al.*, 2007). Maximum number of unfilled grains was found from P_0 (19.4%) whereas minimum from P_1 (5.2%) which was statistically identical with P_2 (5.7%) (Table 02). When *Pteris vittata* was grown along with wheat plant it reduce the arsenic toxicity for wheat plants by rapid accumulating arsenic from soil and resulted less percentage of unfilled grains. *Pteris vittata* remove total arsenic at the end of the 6-week which period can be as high at 68.0% (DeNafo, 2007).

1000-grain weight and yield

1000-grain weight was not varied significantly among the trap plant density while yield was varied significantly among the treatments. However, maximum number of weight found in P_0 (43.4 g) and minimum from and P_2 (42.7 g) which was statically identical P_1 (43.0 g) (Table 02). 1000-grain weights of rice were decreased with increasing of arsenic (Azad *et al.*, 2012) due to the presence of arsenate at a higher concentration (Abedin *et al.*, 2002a). Arsenic at 50 mg/kg or 100 mg/kg soil significantly reduced the thousand-kernel weight in four Chinese variety of wheat (Zhang *et al.*, 2009) which is not supported the findings of the current study. Yield can increases due to small additions of arsenic for corn, potatoes, rye and wheat (Carbonell-Barrachina *et al.*, 1998; Gulz, 1999) while yield may decrease by increasing levels of heavy metals due to phytotoxic effects of heavy metals on plants (Ducsay, 2000). The result of the current study showed that 1000-grain weight increase due to 50-ppm arsenic contamination in soil. Displacement of phosphate ions from the soil by arsenate ions results increase of phosphate availability (Jacobs *et al.*, 1970). Available phosphate may be responsible for increasing 1000-grain weight. Maximum wheat yield plant was found from P_1 (5.7 g per plant) which was statistically identical with P_2 (5.4g per plant) and minimum from P_0 (3.4 g per plant) (Table 02). Using *Pteris vittata* increases the yield per plant by producing more number of filled grains and reducing the number of unfilled grains. *Pteris vittata* reduce arsenic toxicity form soil and accumulate huge amount of arsenic into their fronds and it is possible to use *P. vittata* to remediate arsenic-contaminated soils by repeatedly harvesting its fronds (Ma *et al.*, 2001).

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Table 2. Wheat responses to *Pteris vittata* density at different attributes

Treatments	Filled grains/spike	Unfilled grains/spike	Unfilled grains (%)	Weight of 1000-grain (g)	Yield (g)/plant
P_0	23.5 b	5.7 a	19.4 a	43.4 a	3.4 b
P_1	29.2 a	1.6 b	5.2 b	43.0 b	5.7 a
P_2	28.5 a	1.7 b	5.7 b	42.7 b	5.4 a
LSD0.05	*1.2*	*1.0*	*3.3*	*0.3*	*1.0*
CV%	*1.9*	*4.2*	*4.3*	*0.3*	*8.7*

^{X} P_0 = Control (without *Pteris vittata*), P_1 = 4 *Pteris vittata* per square meter, P_2 = 8 *Pteris vittata* per square meter, ^{Y} In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

Arsenic accumulation in wheat grain and straw

Arsenic accumulation in grain was varied significantly among control treatment and treatments with *Pteris vittata*. Maximum arsenic accumulation in grain was found in P_0 (1.34 ppm) while minimum accumulation P_2 (0.01 ppm) which was statistically identical with P1 (0.02 ppm) (Table 03). Maximum concentration of arsenic in grain (0.16 µg per g) was measured in Protiva variety of wheat when it was cultivated with 40 ppm arsenic per kg soil (Asaduzzman *et al.*, 2010). *Pteris vittata* reduce the arsenic toxicity from the soil and render the arsenic enter into plant (Mayda *et al.*, 2014). As a result, it is possible to reduce arsenic accumulation into food crops by inters planting *Pteris vittata***.** Highest arsenic accumulation of wheat straw was found from P_0 (35.20 ppm) and lowest accumulation of wheat straw was found from P_1 and P_2 (1.17 and 1.07 ppm respectively) which are statistically identical (Table 03). Plant tissues were susceptible in the order of root>straw>grain and the concentrations of arsenic were dependent on variety and treatment levels in polluted soils and statistically maximum concentration of arsenic in root (3.08 µg per g), straw (0.9233 µg per g) and grain (0.16 µg per g) were measured in Protiva variety when it was cultivated with 40 ppm arsenic per kg soil. On the contrary, Gourav contained significantly less amount arsenic in root (2.34 µg per g), straw (0.376 µg per g) and grain (0.04 µg per g) at the same level of arsenic contaminated (Asaduzzman *et al.*, 2010). In current study, *Pteris vittata* uptake arsenic from soil and also reduce arsenic toxicity from soil. Significant differences in arsenic concentration in polluted areas but wheat is used for food or fodder with lowest arsenic concentration (Zhang *et al.*, 2009).

Arsenic accumulation by *Pteris vittata*

Maximum accumulation was found from P_8 (42.00) and minimum accumulation was found from P_4 (41.60) (Table 03). On the other hand protection of arsenic accumulation into wheat was 97.44% and 97.16%. *Pteris vittata* uptake high amount of arsenic. *Pteris vittata* L. is efficient to accumulate 4200 mg per kg As in its aboveground biomass growing in a soil containing 131 mg per kg As (Fayiga *et al.*, 2004). *Pteris vittata* accumulated As in the fronds up to 27,000 mg per As kg dry weight, and the frond As to root As concentration ratio varied between 1.3 and 6.7 (Wang *et al.*, 2002). *P. vittata* reduced soil arsenic by 6.4-13% after three time harvesting (Ma *et al.*, 2008).

Table 03. The arsenic content in grain, straw, total accumulation and protection percentage by wheat plant as affected by different *Pteris vittata* density^Y

Treatments^X	Arsenic accumulation (ppm)			Protection of arsenic accumulation into wheat (%)
	wheat grain	wheat straw	*Pteris vittata*	
P_0	1.34 a	35.27 a		
P_1	0.02 b	1.17 b	41.60	97.16
P_2	0.01 b	1.07 b	42.00	97.44
LSD0.05	*0.07*	*1.25*		
CV%	*6.6*	*4.4*		

^{X} P_0= Control (without *Pteris vittata*), P_1 = 4 *Pteris vittata* per square meter, P_2 = 8 *Pteris vittata* per square meter, ^{Y} In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

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IV. Conclusion

Wheat plant grown with *P. vittata* accumulated only 0.01-0.02 ppm arsenic in grain while 1.34 ppm arsenic accumulation was found in grain without *P. vittata*. So, it can be stated that *P. vittata* might be acted as the trap plant and reduce the arsenic accumulation into wheat about 96.16-97.44%. Further study should be conducted on the arsenic contaminated field to strengthen the findings of the current study.

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