Percentage. However, Arsenate acts as Pi analogue **(Pigna et al 2010).** Phosphate fertilization likely reduces the impact of arsenic toxicity without increase in arsenic concentration in black gram **(Pigna et al 2009).**

Materials and Methods:

The seeds and pots were purchased from local market of Boring road, Patna. 12 pots for different concentration and one pot for control was taken. All experiments were done in triplet. 900g garden soil and 300g organic compost (3:1) was taken in each pot. 25 Black gram seeds were sterilized with 0.1g mercuric chloride for 2mins and washed thoroughly. Presoaked seeds were sown into 0µm (Control), 100µm As, 200µm As with and without 40ppm K_2HPO_4 solution for 3-4 hours. 250ml of arsenic of above concentration was given weakly in each pots for 30 days. The whole setup was left for 7 days under laboratory condition at 16±4°C. Germination % was recorded after 7 days of sowing **(Chauhan et al 2016).**

Germination % was obtained by following formula:

$$\text{Germination \%} = \frac{\textit{(No.of germination seed in pot)/}}{\textit{(Total no.of seeds sown in pot)}} \times 100$$

Observational morphological changes in the plant seedlings:

Length of seedling, fresh and dry weight of sample was recorded on 30th day **(Jasrotia et.al 2014).**

Estimation of photosynthetic pigments

The chlorophyll a and chlorophyll b content from each test sample was estimated on 30^{th} day **(Ritchie et al 2006).** 100mg black gram leaves from different concentration were crushed and 10ml of 80% chilled acetone was added in it. The extract was centrifuged at 2000(rpm) for 10min. The absorbance of filtrate obtained was taken at 645nm and 663nm using UV-VIS spectrophotometer.

The concentration of pigment was calculated by using the formula:

$$\text{Chlorophyll a} = \frac{([12.7\ (A663\text{-}2.69\ (A645))])}{(1000\times W)} \times V$$

$$\text{Chlorophyll b} = \frac{([22.9\ (A645\text{-}4.68\ (A663)]}{(1000\times W)} \times V$$

Here,

A=Absorbance at specific wavelength
V=Final volume of chlorophyll extract
W=Fresh weight of tissue extracted

Protein content of each concentration was assesed using standard protocol **(Larson et al 1986).**

Estimation of Electrolyte Leakage Percentage (ELP)

ELP was calculated by using formula: **(Bajji et al 2002)**

$$\text{ELP} = \frac{(E_2\text{-}E_1)}{E3} \times 100$$

E_1 = Initial Conductivity
E_2 = Middle Conductivity
E_3 = Final Conductivity

Results and Discussion:

Significant changes were observed in the test samples treated with arsenic and potassium phosphate at different concentration. The results are shown in Table 1.

Table 1. Percentage of Germination on 7th day

Sl. No.	Retention Period	Sample Pots With Different Concentration of Arsenic Phosphate	No. of seeds sown	No. of seeds germinated in 3 pots.			Percen-tage Germinati on (%)
				I.	II.	III.	
1.	7 days	Control	25	14	17	23	72
2.		100µm As	25	7	16	23	61.2
3.		200µm As	25	4	8	11	30.4
4.		100µm As+40ppm	25	16	18	15	65.2
5.		200µm As+40ppm	25	10	16	9	46.4

The given Table 1 shows that germination percentage of seeds which is lowest in sample with highest concentration of Arsenic. This result is similar to work done by (Chauhan et al 2016) Germination % decreases with increasing the applied arsenic concentration. In control, germination rate is 72% and it decline to 61.2% in 100μm and 30.4% at 200μm. However, phosphate applied seed concentration increases from 61.2% - 65.2% (100μm+P) and 30.4% - 46.4% (200μm+P).

Table 2. Effect on root, shoot, dry weight and fresh weight of black gram seedlings

Sl. No.	Sample pots with different concentration of Arsenic and Phosphate	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
1.	Control	40.66±2.37	9.5	0.94±0.56*	0.133g±0.030*
2.	100μm As	39.26±1.56	5.8±0.2	1.15±0.07*	0.131±0.001*
3.	200μm As	0	0	0	0
4.	100μm+p	42.7±1.6	11.4±2.8	1.30±0.48*	0.162±0.001*
5.	200μm+p	8.4±1.24	3.2±2.1	0.32±0.11	0.053±0.002*

P=40ppm K_2HPO_4; value are means of 3 replicates ± SE* Data significant at P<0.05.Multiple comparison versus control group (Holm Sidak method) over all significance level = 0.05.

Table 2 shows that length of root and shoot of black gram decreases with higher arsenic dose as calculated by Jasrotia et.al (2014). It is observed that shoot length of control is 40.66cm which decreases up to 39.26cm (100μm) and 0cm at 200μmAs concentration.

The length of root at control is 9.5 cm which is decreasing at 5.8 cm (100μm) and 0 cm at 200μm. However, black gram treated with As+P is less severe as it increase the length of shoot from 39.26cm – 42.7cm (100μm+P); 0cm – 8.4cm (200μm+P) and length of root from 5.8cm – 11.4 cm (100μm+P); 0cm – 3.2cm (200μm+P). Fresh weight and dry weight of arsenic decreases with increasing arsenic dose.

Table 3. Estimation of pigment level of the test samples

Sl. No.	Treatment	Absorbance at different wavelength	Absorbance of different wavelength	Chlorophyll -a (mg/g few of tissue)	Chlorophyll-b (mg/g few of tissue)	Total chlorophyll (mg/g few of tissue)
		663nm	645nm			
I.	Control	0.391	0.399	0.35	0.65	1
II.	100μm	0.340	0.356	0.30	0.59	0.89
III.	200μm	0	0	0	0	0
IV.	100μm+p	0.377	0.399	0.33	0.61	0.94
V.	200μm+p	0.360	0.366	0.32	0.60	0.92

Table 3 shows that concentration of arsenic increased in water, the amount of chlorophyll content decreases day by day. On 30^{th} day there is huge variation seen in all pots. It was found that chlorophyll content decrease in high concentration of As because increase in arsenic level change chloroplast shape with concaving membrane bending and partial destruction (Miteva and Merakchiyska, 2002).The chlorophyll content in black gram is estimated highest in plants of control showing chlorophyll a and b i.e., 0.35mg, 0.65mg and decreases chlorophyll a and b content i.e., 0.30mg, 0.59mg (100μm) and no chlorophyll (200μm). However, black gram treated with As+P increase chlorophyll a and b content 1.e., 0.33 mg , 0.61mg (100μm + P) and 0.32mg, 0.60mg (200μm+P).

Table 4. Estimation of protein Using UV-VIS Spectrophotometer

Sl. No.	Treatment	Wavelength (nm)	Absorbance (mg/100mg tissue)	Concentration mg/ml
1.	Control	750	0.204	593
2.	100 μm	750	0.122	355
3.	200 μm	750	0	0
4.	100 μm + P	750	0.138	386
5.	200 μm + P	750	0.063	178

In Table 4, it shows that increased concentration of arsenic decreases the protein content. The protein content in *Vigna mungo* is estimated highest in control showing absorbance of 0.204mg and decreases to 0.122mg (100µm) and 0mg at 200µm. When As+P treated then Absorbance of protein increases to 0.138mg (100µm+P) and 0.063mg (200µm+P) (Larson et al., 1986).

Table 5. Determination of Electrolyte Leakage percentage (ELP)

Sl. No.	Treatment	Electrolyte Leakage Value			ELP (%)
		E1	E2	E3	
1.	0	0.120	0.155	0.146	23.97
2.	100µm	0.324	0.372	0.337	14.24
3.	100µm+p	0.378	0.364	0.433	3.23

As shown in Table 5, the arsenic induced membrane damage from an increased electrolyte leakage from leaves (Bajji et al., 2002). The ELP in control showing 23.97% when arsenic concentration increase ELP up to 14.24% in 100µm + P. However, plant treated with As+P decreases ELP level from 14.24% to 3.23% in 100µm+P.

Conclusion:

The present research work shows that the different concentration of Arsenic applied either singly or in combination with phosphate affects the metabolism and physiological properties of *Vigna mungo.* Arsenic plays a negative role as growth inhibitor rather than growth promoter. In black gram arsenic adversely affect the morphological growth and yield of plants. From the present research work by comparing all the pots of 0µm, 100µm, 200µm, 100µm+P and 200µm+P concentration, it was concluded that plant treated only with high arsenic concentration showed the maximum decrease in root and shoot length, chlorophyll content, germination percentage and protein content. This clearly suggests that black gram is quite sensitive towards arsenic toxicity. Comparing black gram with other plant like rice, wheat, tomato, maize etc., seedling have also shown reduction in their germination%, biomass of root/shoot elongation when exposed to arsenic. However, phosphate application ameliorated to a large extent, the damaging effects caused by arsenic toxicity. Arsenic and Phosphate compete for same uptake carrier, it is seen that Arsenic toxicity is lower under high Phosphate condition.

Acknowledgment:

We express our great sense of gratitude to our principal, Dr. Sister M. Rashmi. A.C, giving us the opportunity to do this research work. We are also grateful to Dr. Pinky Prasad, Head of the Department of Botany, Patna Women's College, Patna.

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