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Germination Potential and Seedling Performance of Green Gram in Arsenic Contaminated Hydroponic Culture

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Abstract

In the present study, short term hydroponic culture experiments using cotton soaked with sodium arsenite and arsenic contaminated poultry dung suspension have been conducted on green gram. In poultry dung average pH, conductivity, organic carbon, nitrate, phosphate and arsenic content were found as 7.25± 0.59, 1.256± 0.08 mS, 218.4± 32.75mg/gm, 2.57± 0.17mg/gm, 21.44 ±4.47mg/gm, and 0.038±0.005mg/gm respectively. In sodium arsenite contaminated culture with arsenic concentration ranging from 0.5 to 10 ppm, maximum seed germination was found in 0.5 ppm As (98.88%) and minimum in 10 ppm (30 %). Germination index (GI), relative growth index (RGI) and quality index (QI) also showed a maximum of 96.61%, 95.54% and 0.234% respectively at 0.05 ppm As in arsenic contaminated culture. Poultry dung suspension (PDS) culture showed 100% seed germination in 1%, 3% and 5% poultry dung suspension (PDS) and in 7%,10%, 15%, 20%, 25% and 50% the germination was found as 98.88%, 96.66%, 86.66%, 73.33%, 71.11% and 53.33% respectively with a maximum of 99.12% GI, 98.21% RGI and 1.44% QI at 1% PDS respectively. Thus PDS culture was favourable for germination and seedling potential of gram

Keywords: As, poultry dung, hydroponic, germination, green gram.

Introduction

Arsenic (As) is a toxic metalloid found in rocks, soil, water, sediments and air. It enters into the terrestrial and aquatic ecosystems through a combination of natural processes such as weathering reactions, biological activity, and volcanic emissions as well as a result of anthropogenic activities. Excessive use of As-based pesticides and indiscriminate disposal of domestic (sewage) and industrial (timber, tannery, paints, electroplating, etc.) wastes, as well as mining activities, have resulted in widespread As contamination of soils and waterways. Arsenic in terrestrial and aquatic ecosystems attracts worldwide attention primarily because of its adverse impact on human health. The general population may be exposed to as from air, food, and water[1]. Of the various sources of as in the environment, water probably poses the greatest threat to human health[2]. Arsenic is a compound that is extremely hard to convert to water soluble or volatile products. The fact that arsenic is naturally a fairly mobile component[3] basically means that large concentrations are not likely to appear on specific site. This is a good thing, but the negative side to it is that arsenic pollution becomes a wider issue[4], because it easily spreads. In plants, as generally interfere in food mobilization in phloem by adhering to the cell walls[5]. So there may be lack of nutrition, causing retardation in growth. As act as uncoupler in oxidative phosphorylation. Thus it interferes in ATP / ADP interconversion causing problem in energy supply to the growing cells. Arsenate and Arsenite inactivate fumarase, a key enzyme in kreb's cycle and thus causes inhibition of the cycle. It leads to disruption in metabolism. This disruption in metabolism lead to growth inhibition. As has been found as the main reason for chlorosis[6] which inhibit the growth, by indirectly affecting photosynthesis. As is responsible for altering the apical dominance of plants[5] which stops plant growth. Arsenic, in the forms of roxarsone and Arsanilic acid is an additive in the feed of conventionally-raised broilers. It is used to control protozoan parasites known as coccidian and to enhance weight gain. In soil, chemical and microbial reactions readily transform roxarsone into inorganic forms of arsenic. These inorganic forms are then subject to a variety of chemical and biological reactions in the soil. Soil mineralogy, soil moisture, soil pH, and microbial reactions all determine arsenic mobility, its uptake by plants, and its toxicity. Plant uptake is one of the major path ways by which metal soils enter the food chain. The food-chain plants might absorb enough amounts of heavy metals to become a potential health hazard to consumers[17].

Material and Methods

Analysis of physiochemical characteristics of poultry dung: For analysis of physico-chemical parameters and arsenic content of poultry dung as well as in poultry feed, 5 different poultry farms were selected from Sambalpur-Bargarh area of Odisha state. Those are i. Indian Poultry Farm, Bargarh, ii. Katapali poultry farm, A. Katapali, iii. Kunal Poultry Farm, Chipilima, iv. M/S Parimal Poultry Shop, Burla and v. Sri Bhenkateswar Poultry Farm, Attabira.

Analysis of pH, conductivity, organic carbon, nitrate, phosphate and arsenic content of poultry dung were made following established methods. pH and conductivity were measured using digital pH meter and conductivity meter with automatic temperature compensation, calibrated with calibration solutions[7]. Organic Carbon was determined by Walkey-Black titration method as described by Walkey-Black[8]. Nitrate was estimated by phenoldisulphunic acid method[9] and phosphate by stannous chloride method[10]. Estimation of arsenic was done by silver diethyldithiocarbamate method[11].

Experimental Design (Hydroponic culture): A good variety of green gram seed (*Vigna radiata*) was collected from Goshala seed store, Sambalpur. The seeds were air dried and stored at room temperature until use. Uniform sized seeds of green gram were subjected to surface sterilization with 0.2% $HgCl_2$ for 2 minutes and repeatedly washed thoroughly with distilled water to remove all the traces of mercuric chloride[12, 18]. The seeds were then placed on sterilized Petri dishes (15 × 20 cms) at equal distance and were treated with equal volume of different concentration of arsenic (As) solution and different poultry dung suspension. Seeds treated with distilled water were maintained as control. Three replicates were kept under diffused light at room temperature (28 ± 1^0C). The most important products which are obtained from this crop are grains, oil, pulse, milk, curd, sweets, soya protein, neutrinugget, soya sauce etc[12]. The initial appearance of radical was taken as indicative of germination. Percentage germination was calculated as per the method by Ferrara et al.[13] as Seed germination % = (Number of germinated seeds/ Total no. of seeds) ×100. Germination Index (GI) of the 15^{th} day old seedling was calculated as per the method of Ferrara et al. [13] using the formula as

$$\text{Germination Index} = \{(Gt \times Lt)/ (Gc \times Lc)\} \times 100$$

Where, Gt=percentage of seed germination in treated set, Gc= percentage of seed germination in control set, Lt = root length in the treated seedling and Lc = root length in control seedling. Relative Growth Index (RGI) was calculated on dry shoot weights according to the formula by Ferrara et al.[13] as

$$\text{Relative Growth Index} = Wt/Wc \times 100$$

Where, Wt = dry shoot weight of treated plants and Wc = dry shoot weight of control plants). Quality index (QI) was calculated as Quality Index = TW/ (H/D) + (SW+RW)[14]. Where, TW= Total Seedling Dry weight, H= Seedling Height, D= Collar Diameter, SW= Shoot Dry weight and RW= Root Dry weight. Growth, in terms of morphological changes studies in 15 days old seedlings were carried out following the method described by Kemp[15], ICAR[16] and Ferrara et al. [13].

Results and Discussion

Physicochemical characteristics of poultry dung and Arsenic content of poultry feed: The physico-chemical characteristics of poultry dung collected from 5 different poultry farms were analyzed (table 1). The poultry dung was characterized by 1.25± 0.08 mS electrical conductivity, 2.57± 0.17mg/g nitrate, 21.44±4.46mg/g phosphate and 218.4±32.75mg/g organic carbon with a pH of 7.25± 0.59. The arsenic content of poultry feed and poultry dung were found to be 0.018±0.006 mg/g and 0.038±0.005 mg/g respectively. The high EC values of poultry dung may be attributed to higher salt levels of nitrate and phosphate. The pH of poultry dung was found to be slightly alkaline. Arsenic content was found higher in dung than feed.

Germination and growth: In sodium arsenite contaminated culture the maximum seed germination of gram in control was 98.88% on 15^{th} day, 96.67% at10th day with 73.33.6% germination within 24 hour. In the lowest arsenic concentration of 0.5 ppm, germination was 53.33.66% within 24 hour and a maximum 96.76% on 12^{th} day. No germination was observed above 10 ppm concentration of As contaminated culture table-2. The germination results revealed that the increasing concentrations decreased germination and seedling growth in all the sets[20]. The extent of decrease varied with cultivars and salt concentrations. Performance of seedlings on 15^{th} day of the growth in As contaminated hydroponic culture table-3 reveals a consistent retardation in height, collar diameter, shoot and root dry weight, total seedling dry weight over control with very negligible shoot weight and root weight (< 0.001g) beyond 4ppm concentration. Germination and growth were quantified in the form of Germination index and Relative Growth Index. A maximum of 62.99% GI and 77.77% RGI were recorded at 0.5 ppm As contaminated culture with a systematic decrease with increase in As concentration reaching a minimum RGI of 11.11% at 3 ppm. The Quality Index was found to be highest (0.0012) in control set followed by 0.0009 in 0.5 ppm arsenic contaminated culture. One way ANOVA reveals a significant difference in growth parameters between concentration ($P<0.05$). The overall effect of arsenic on plants is the eventual shrinkage of leaf size, which leads to death of the leaf, and finally the plant. Arsenic may also cause reduced ATP and growth regulators in plants and ceased the germination potential[23].

Table-1
Physical and chemical characteristics of poultry dung and Arsenic content in poultry feed

Samples	pH	Conductivity (mS)	Organic Carbon Content (mg/g dry wt)	Nitrate (mg/g dry wt)	phosphate (mg/g dry wt)	As content in poultry dung (mg/g dry wt)	As content in poultry feed (mg/g dry wt.)
A	7.68	1.378	252	2.808	26.4	0.036	0.019
B	7.01	1.191	174	2.352	15.2	0.041	0.026
C	7.89	1.298	198	2.508	19.2	0.03	0.009
D	6.39	1.203	222	2.544	21.6	0.039	0.021
E	7.31	1.212	246	2.664	24.8	0.044	0.015
SD MEAN	7.25±0.59	1.256±0.08	218.4±32.75	2.575±0.17	21.44±4.46	0.038±0.005	0.018±0.006

Table-2
Germination Potential of Green gram in hydroponic Arsenic contaminated culture

Conc.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Control	73.33	80	90	90	90	90	90	93.33	96.67	96.67	96.67	97.72	98.88	98.88	98.88
0.5ppm	53.33	63.33	63.33	73.33	73.33	76.67	80	83.33	93.33	93.33	96.76	96.76	97.72	97.72	97.72
1ppm	40	43.33	43.33	53.33	53.33	56.67	60	66.67	70	83.33	83.33	83.33	88.88	88.88	88.88
1.5ppm	46.67	53.33	55.67	60	63.33	66.67	66.67	70	76.67	80	81.21	81.21	81.21	81.21	81.21
2ppm	33.33	46.67	53.33	60	63.33	66.67	70	70	76.67	76.67	76.67	78.18	78.18	78.18	78.18
2.5ppm	40	36.67	43.33	50	53.33	53.33	56.67	63.33	70	70	70	73.33	73.33	73.33	73.33
3ppm	36.67	43.33	43.33	46.67	46.67	50	53.33	56.67	66.67	66.67	67.27	67.27	71.11	71.11	71.11
3.5ppm	36.67	43.33	46.45	46.67	47.23	47.23	47.23	54.53	54.53	54.53	56.67	58.88	63.33	68.28	68.28
4ppm	35.55	44.14	44.14	44.14	46.26	46.26	47.23	47.23	47.23	51.11	51.11	57.74	57.74	63.33	63.33
4.5ppm	33.33	33.33	42.12	42.12	42.12	44.44	44.44	47.23	48.18	48.18	48.18	55.55	58.38	61.11	61.11
5 ppm	31.11	31.11	34.32	34.32	36.67	36.67	36.67	40	43.33	46.67	46.67	48.28	48.28	48.28	48.28
10 ppm	0	0	0	0	0	0	0	0	0	0	0	27.76	27.76	30	30

Two-way ANOVA, $F1=348.6461$, $F_2=22.658$, $p<0.05$

Table-3
Performances of seedling on 15th day in Arsenic contaminated culture

CONC.	H (cm)	D (mm)	RW (g)	SW (g)	TW (g)	QI	GI(%)	RGI(%)
Control	7.9	8	1.324	1.025	2.349	0.234	nil	nil
0.5 PPM	6.6	7.4	1.210	0.8	2.01	0.213	96.61	95.54
1 PPM	5.8	6.1	0.98	0.61	1.59	0.208	93.33	86.25
1.5 PPM	5.4	5	0.81	0.54	1.35	0.198	90.01	84.61
2 PPM	3.9	4	0.74	0.4	1.14	0.178	88.81	78.75
2.5 PPM	2.2	3.2	0.62	0.32	0.94	0.164	84.44	67.56
3 PPM	1.1	3	0.49	0.24	0.73	0.152	81.23	61.26
3.5 PPM	0.8	1.8	0.21	0.11	0.32	0.146	79.67	58.47
4PPM	0.6	1	0.16	0.02	0.18	0.131	76.66	56.66
4.5PPM	0.4	0.6	0.11	0.01	0.021	0.122	68.26	44.43

Where, H= Seedling Height; D= Collar Diameter; SW= Shoot Dry weight; RW= Root Dry weight; TW= Total Seedling Dry weight; GI=Germination Index; RGI=Relative Growth Index; QI=Quality Index

Poultry dung suspension (PDS) culture showed 100% seed germination on 5th day and 7th day in 1% PDS and 5% PDS respectively and the germination was delayed with increase in concentration. However, even at 50% PDS, the germination recorded was 86.66% table-4. Table-5 presents the performance of seedlings grown in PDS culture on 15th day of the growth. The analysis reveals very negligible effect of PDS on growth parameters with 111.95% GI at 1%PDS where as Relative Growth Index (114.28%) as well as Quality Index (0.008) was maximum in 50% PDS. One way ANOVA reveals no significant difference in growth parameters between concentration (P<0.05).

Table-4
Germination Potential of Green gram in Poultry dung suspension culture

CONC.	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
Control	86.66	93.33	100	100	100	100	100	100	100	100	100	100	100	100	100
1% PD	20	30	83.33	100	100	100	100	100	100	100	100	100	100	100	100
3% PD	30	46.66	83.33	86.66	100	100	100	100	100	100	100	100	100	100	100
5% PD	83.33	83.33	93.33	93.33	93.33	96.66	96.66	97.76	97.76	99.99	100	100	100	100	100
7% PD	83.33	86.66	90	93.33	93.33	93.33	93.33	96.66	96.66	97.76	97.76	98.88	98.88	98.88	98.88
10% PD	76.66	83.33	83.33	86.66	86.66	86.66	93.33	93.33	93.33	93.33	93.33	96.66	96.66	96.66	96.66
15%PD	76.66	76.66	76.66	76.66	76.66	76.66	83.33	83.33	83.33	83.33	83.33	86.66	86.66	86.66	86.66
20%PD	71.11	71.11	71.11	71.11	71.11	71.11	71.11	71.11	73.33	73.33	73.33	73.33	73.33	73.33	73.33
25%PD	69.96	69.96	69.96	69.96	69.96	69.96	69.96	69.96	69.96	69.96	71.11	71.11	71.11	71.11	71.11
50%PD	51.11	51.11	51.11	51.11	51.11	51.11	51.11	51.11	51.11	51.11	51.11	51.11	51.11	53.33	53.33

Two-way ANOVA, F1=187.985, F_2=122.677, $p<0.05$

Table-5
Performances of Seedling on 15th Day in Poultry Dung Suspension Culture

Treatment	H (cm)	D (mm)	RW (g)	SW (g)	TW (g)	QI	GI(%)	RGI(%)
Control	29.3	10.2	1.91	1.76	3.67	1.62	nil	nil
1%	26.1	9.1	1.66	1.02	2.68	1.44	99.12	98.21
3%	24.5	8.2	1.11	0.949	2.059	1.34	97.42	95.75
5%	22.2	8.1	0.97	0.823	1.793	1.29	95.41	90.15
7%	20.3	7.3	0.942	0.766	1.708	1.28	93.42	89.53
10%	18.7	7.2	0.862	0.721	1.583	1.24	90	86.66
15%	15.9	7.1	0.762	0.694	1.456	1.19	86.66	80.54
20%	15.1	6.1	0.623	0.554	1.177	1.15	82.68	78.54
25%	13.2	6.2	0.603	0.423	1.026	1.02	80	72.22
50%	9	4.2	0.584	0.411	0.995	0.12	72.21	64.52

Where, H= Seedling Height; D= Collar Diameter; SW= Shoot Dry Weight; RW= Root Dry weight; TW= Total Seedling Dry weight; GI=Germination Index; RGI=Relative Growth Index; QI=Quality Index

It has been found that the germination and growth performances of plants are severely affected by various concentrations of As in arsenic contaminated culture. The germination and growth started retarding with increase in concentration of As. The seed size is a considerable and significant factor in the germination and early stage of plant growth[21]. With increase in the concentrations of As in ppm, the growth slowdown, and in high concentrations the growth was ceased. It has been also found that some plants are growing despite the high concentrations of As, but their growth rates are negligible. However in case of poultry dung suspension culture, the germination and growth of plant is not much affected at 1% and 5% concentration as poultry manure contains the essential plant nutrients that are used by the plants but above 10% the germination and growth were effected probably due to higher concentration of arsenic in poultry manure. Thus, Poultry manure as a fertilizer for crops may provide a portion or all of the plant requirements but it should be applied with caution as applying more amounts of poultry manure means adding more amount of arsenic to the soil.

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