The Bioscan 7(2) : 299-302, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

GERMINATION POTENTIAL AND SEEDLING PERFORMANCE OF YELLOW MUSTARD IN ARSENIC CONTAMINATED HYDROPONIC CULTURE

LAKSHMIKANTI BHOI, DULLAV BHOSAGAR, P. C. MISHRA*
Department of Environmental Sciences (Auto),
Sambalpur University, Jyoti Vihar - 768 019, Orissa, INDIA
E-mail: profpcmishra@gmail.com

KEY WORDS
As, Poultry dung
Hydroponic
Germination
Mustard

Received on :
22.02.2012

Accepted on :
18.05.2012

***Corresponding author**

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 yellow mustard. In poultry dung average pH, conductivity, organic matter, nitrate, phosphate and arsenic content were found as 7.25± 0.59, 1.25± 0.08mS, 218.4± 32.7mg/g, 2.57± 0.17mg/g, 21.44 ±4.47mg/g, and 0.038±32.7mg/g respectively. In sodium arsenite contaminated culture with arsenic concentration ranging from 0.5 to 10ppm, maximum seed germination was found in 0.5ppm As (83.33%) and minimum in 10ppm (10 %). Germination Index (GI), Relative Growth Index (RGI) and Quality Index (QI) also showed a maximum of 62%, 77.77% and 0.0108% respectively at 0.05ppm As in arsenic contaminated culture. Poultry dung suspension (PDS) culture showed 100% seed germination in 1% and 5% poultry dung suspension (PDS) and in 10%, 25% and 50% the germination was found as 93.33%, 93.33% and 86.66% respectively with a maximum of 111.95% GI, 114.28% RGI and 0.366% QI at 1%, 5% and 10% PDS respectively. Thus PDS culture was favourable for germination and seedling potential of mustard.

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 (Adriano, 2001). Of the various sources of As in the environment, water probably poses the greatest threat to human health (Smedley and Kinniburgh, 2002).

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 (Pershagen, 1981) 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 (Enterline and Marsh, 1980), because it easily spreads. In plants, As generally interfere in food mobilization in phloem by adhering to the cell walls (Chen *et al.,* 2002). 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 (Gupta and Charles, 1999) which inhibit the growth, by indirectly affecting photosynthesis. As is responsible for altering the apical dominance of plants (Chen *et al.,* 2002) which stops plant growth. The inhibition of germination and root growth was noticed by different researcher at higher concentrations of Arsenic in wheat (Panda and Patra, 1997), Cowpea (Lalitha *et al.,* 1999), Pea (Chugh and Sawhney, 1996) and cotton (Shrivastava *et al.,* 1997).

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.

Mustard is cultivated in most part of the world because mustard seed is a rich source of oil and protein. The present investigation has been carried out to find out the effect of different concentrations of arsenic (in the form of sodium arsenite and poultry dung containing roxarsone and arsanilic acid) on seed germination and growth parameters of mustard in hydroponic condition.

MATERIALS 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 (Jackson, 1973). Organic Carbon was determined by Walkey-Black titration method as described by Walkey-Black, (1934). Nitrate was estimated by phenoldisulfonic acid method (Harper, 1924) and phosphate by stannous chloride method (Olsen *et al.,* 1954). Estimation of arsenic was done by silver diethyldithiocarbamate method (Ballinger *et al.,* 1962).

Experimental design (Hydroponic culture)

A good variety of mustard seed (*Brassica compestris*) was collected from Goshala seed store, Sambalpur. The seeds were air dried and stored at room temperature until use. Uniform sized seeds of mustard 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. The seeds were then placed on sterilized Petri dishes (15 × 20cms) 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 ± 1ºC). The initial appearance of radical was taken as indicative of germination (Behera and Mishra, 1982). Percentage germination was calculated as per the method by Ferrara *et al.* (2003) as Seed germination % = (Number of germinated seeds/ Total no. of seeds) ×100. Germination Index (GI) of the 15th day old seedling was calculated as per the method of Ferrara *et al.* (2003) using the formula as Germination Index = {(Gt × Lt)/ (Gc × Lc)} × 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.* (2003) as Relative Growth Index = Wt/Wc × 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) (Dickson *et al.* 1960). (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 (1960), ICAR (1997) and Ferrara *et al.* (2003).

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 ± 0.08mS 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 mustard in control was 96.67% on 14th day, 93.33% at10th day with 66.6% germination within 24h. In the lowest arsenic concentration of 0.5ppm, germination was 26.66% within 24h and a maximum 83.33% on 12th day. At As concentration of 10ppm, the germination was delayed with only 3.33% on 8th day and maximum 10% on 14th day. No germination was observed above 10ppm concentration of As contaminated culture (Table 2). Performance of seedlings on 15th 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 3ppm. 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 ($p < 0.05$).

Table 1: Physical and chemical characteristics of poultry dung and as 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
	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 mustard 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	66.6	66.6	66.6	80	80	90	90	90	90	93.33	93.33	93.33	93.33	96.67	96.67
0.5 PPM	26.66	26.66	26.66	53.33	56.66	60	60	60	80	80	80	83.33	83.33	83.33	83.33
1 PPM	20	26.66	26.66	26.66	36.66	53.33	53.33	53.33	60	60	63.33	63.33	63.33	63.33	63.33
1.5 PPM	16.66	16.66	16.66	16.66	26.66	40	40	40	40	43.33	43.33	53.33	53.33	60	60
2 PPM	10	13.33	13.33	13.33	26.67	26.67	26.67	40	43.33	43.33	43.33	43.33	43.33	56.67	56.67
2.5 PPM	0	10	10	10	23.33	23.33	26.67	26.67	26.67	36.67	36.67	36.67	36.67	36.67	36.67
3 PPM	0	3.33	6.67	6.67	13.33	16.67	16.67	23.33	23.33	26.6	26.6	26.6	26.6	26.6	26.6
4 PPM	0	0	6.67	6.67	13.33	13.33	16.67	16.67	16.67	16.67	16.67	16.67	16.67	16.67	16.67
5 PPM	0	0	0	0	0	0	0	6.66	6.66	6.66	10	10	13.33	13.33	13.33
6 PPM	0	0	0	0	0	0	3.33	3.33	6.67	6.67	6.67	6.67	13.33	13.33	13.33
8 PPM	0	0	0	0	0	0	3.33	3.33	3.33	3.33	6.67	10	10	10	10
10 PPM	0	0	0	0	0	0	0	3.33	3.33	3.33	3.33	6.66	6.66	10	10

(Two-way ANOVA, $F1 = 228.442$, $F_2 = 26.891$, $p < 0.05$).

Table 3: Performances of seedling on 15th day in arsenic contaminated culture

CONC.	H (cm)	D (mm)	SW (g)	RW (g)	TW (g)	QI	GI(%)	RGI(%)
Control	4.9	6	0,009	0.004	0.012	0.0012	-	-
0.5 PPM	3.6	6	0.007	0.002	0.009	0.0009	62.99	77.77
1 PPM	2.8	5	0.006	0.001	0.007	0.0006	42.83	66.66
1.5 PPM	2.4	5	0.004	0.001	0.005	0.0005	31.03	44.44
2 PPM	1.9	4	0.004	0.001	0.005	0.0005	26.29	33.33
2.5 PPM	1.2	3	0.003	<0.001	0.003	0	11.67	22.22
3 PPM	1.1	3	0.002	<0.001	0.002	0	8.4	11.11
4 PPM	0.8	2	0.001	<0.001	0.001	0	4.64	0
F value	67.974*	7.591*	24.489*	12.75*	33.549*	17.437*	14.348*	19.32*

* Significant (One-way ANOVA, $p < 0.05$)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 ($p < 0.05$).

As revealed from As analysis of poultry dung, with an average arsenic content of 0.038mg in 1g of poultry dung, one percent poultry dung suspension contains around 380ppm As. Even at 50% PDS with almost 19000ppm of Arsenic content had no significant effect on germination and seedling performance. Where as in Arsenic contaminated culture no significant germination or seedling performance was achieved beyond 2ppm. This indicates that Arsenic present in poultry dung are mostly in immobile form and do not interfere in plant growth.

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

Table 4: Germination potential of mustard 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	93.33	100	100	100	100	100	100	100	100	100	100	100
5% PD	30	46.66	83.33	86.66	96.66	96.66	100	100	100	100	100	100	100	100	100
10% PD	46.66	83.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33
25% PD	60	86.66	90	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33	93.33
50% PD	76.66	83.33	83.33	86.66	86.66	86.66	86.66	86.66	86.66	86.66	86.66	86.66	86.66	86.66	86.66

(Two-way ANOVA, $F1 = 2.84$, $F_2 = 8.549$, $p < 0.05$).

Table 5: Performances of seedling on 15th day in poultry dung suspension culture

CONC.	H (cm)	D (mm)	SW (g)	RW (g)	TW (g)	QI	GI (%)	RGI (%)
Control	9.1	6	0.009	0.003	0.012	0.0006	-	-
1% PD	8.2	6	0.007	0.002	0.009	0.0005	111.95	100
5 % PD	7.2	5	0.004	0.001	0.005	0.0003	105.43	57.14
10% PD	8.1	6	0.007	0.002	0.009	0.0005	109.17	100
25% PD	7.9	6	0.006	0.001	0.007	0.0004	102.09	85.71
50% PD	8.5	7	0.008	0.003	0.011	0.0008	89.73	114.28
F value	12.814*	0.9**	8.9*	3.6*	9.362*	8.543*	4.755*	19.724*

* Significant (One-way ANOVA, $p < 0.05$) 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

germination and growth started retarding with increase in concentration of As. 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.

REFERENCES

Adriano, D. C. 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals, 2nd Edition, Springer, New York.

Ballinger, D. C., Lishaka, R. J. and Gales, M. E. 1962. Application of Silver Diethyldithiocarbamate method to determination of Arsenic. *J. Amer. Water Works Ass.* **54:** 1424.

Behera, B. K. and Mishra, B. N. 1982. Analysis of effect of industrial effluent on growth and development of rice seedlings. *Environ. Res.* **28:** 10-20.

Chen, T., Wei, C., Huang, Z., Huang, Q., Lu, Q. and Fan, Z. 2002. Arsenic hyper accumulator *Pteris vittata* L. and its arsenic accumulation. *Chinese Science Bulletin.* **47:** 902-905.

Chugh, L. K. and Sawhney S. K. 1996. Effect of cadmium on germination amylases and rate of pea respiration of germinating pea seeds. *Environ. Poll.* **19**:107-124.

Dickson, A. A., Leaf, L. and Hosner, J.F. 1960. Quality appraisal of white spruce and white pine seedlings stock in nurseries. *Forestry Chronicle.* **36:** 10-13.

Enterline, P. E. and Marsh, G. M. 1980. Mortality Studies and Smelter Workers. *American J. Industrial Medicine.* **1:** 251-259.

Ferrara, G., Brunetti, G., Senesi, N., Mondelli, D. and Ghezza, V. L. 2003. Total and potentially phytotoxic metals in South Eastern. *Italian Soils, Food, Agriculture and Environment.* **2:** 279-286.

Gupta, S. L. and Charles, W. 1999. Copper uptake and inhabitation of growth, photoshythetic pigments and macromolecules in the *cyanobacterium Anacystis* ridulans photosynthetica. **20:** 447-452.

Harper, H. J. 1924. The accurate determination of nitrates in soils. Phenoldisulfonic acid method. *Ind. and Eng. Chem.* **16:** 180-183.

ICAR (Indian Council of Agricultural Research) 1997. In, Handbook of Agriculture, Directorate of Publications and Information on Agriculture, Krishi Anusandhan Bhavan, Pusa, New Delhi.

Jackson, M. L. 1973. Soil Chemical Analysis. Prentice Hall India. p.498.

Kemp, C. D. 1960. Method of estimating the leaf area of grasses from linear measurements. *Ann. Bot.* **24:** 491-499.

Lalitha, K., Balasubrahmanian, N. and Kalavathy, S. 1999. Studies of impact of chromium on *Vigna unguiculata* (L.) Walp. var. (Long). *J. Swamy Bot.* **16:** 17-20.

Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean, L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Cire, **939:** U. S. Gov. Print. Office, Washington D.C. pp. 1-19.

Panda, S. K. and Patra, H. K. 1997. Physiology of chromium toxicity in plants. *Rev. Plant Physiol. Biochem.* **24(1):** 10-17.

Pershagen, G. 1981. The Carcinogenicity of Arsenic. *Env. Health Perspect.* **40:** 93-100.

Shrivastava, M. K., Jain, K. C. and Jain, S. 1997. Studies on effect of phosphorus application in cotton (*Gossypium hirsutum* L.). *Adv. Plant Sci.* **12(1):** 279-281.

Smedley, P. L. and Kinniburgh, D. G. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry.* **17:** 517-568.

Walkley, A. and Black, I. A. 1934. Determination of organic carbon in soil. *Soil Science.* **37:** 29-38.