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EFFECTS OF NITROBENZENE ON GROWTH OF TOMATO PLANTS AND ACCUMULATION OF ARSENIC

Mithila Deb, Sajal Roy and S.M. Imamul Huq*

Bangladesh-Australia Centre for Environmental Research, Department of Soil, Water and Environment, University of Dhaka, Dhaka-1000, Bangladesh.

Abstract

The experiment was carried out to study the effect of nitrobenzene (a plant growth regulator) on the growth, nutrient contents and accumulation of arsenic (As) in tomato plants (*Solanum lycopersicum*). Seven different treatments *viz.*, control (C), nitrobenzene only (N), full recommended dose of fertilizer (FRD), ½ recommended dose of fertilizer (½ RD), ½ recommended dose of fertilizer plus nitrobenzene (½ RD+N), ¾ recommended dose of fertilizer (¾ RD), ¾ recommended dose of fertilizer plus nitrobenzene (¾ RD+N) were applied under both arsenic free and arsenic treated conditions. The growth increased due to nitrobenzene application. The highest growth was found with ¾ recommended fertilizer dose with nitrobenzene. The height and fresh matter production of tomato plants were improved in the presence of nitrobenzene in all cases. ¾ RD+N showed the highest accumulation of nitrogen (N) and potassium (K) in shoot and root in both As free and As treated soils. The highest accumulation of phosphorus (P) in shoot and root of As free soils was found in ¾ RD+N. In As non-treated plant samples, the accumulation of sulfur (S) in shoot was found to be at the highest in ½ RD+N, whereas the highest accumulation in root was found in FRD. The highest accumulation of As in shoot and root was found in FRD for As treated soils.

Key words: Accumulation, arsenic, balanced fertilizer, nitrobenzene.

Introduction

Nitrobenzene is an aromatic synthetic nitro compound widely used in industry which can lead to environmental pollution. While the toxicity and carcinogenicity of nitrobenzene on humans and animals have been studied, less is known about its effects on plant growth. However, the susceptibility of plants to nitrobenzene varies with plant species (Farlane *et al.* 1990).

Nitrobenzene is a combination of nitrogen and plant growth regulators that act as plant energizer, flowering stimulant and yield booster (Aziz and Miah 2009). Nitrobenzene produces best results in combination with plant growth regulators, which have capacity to increase flowering in plant and also prevent flower shedding. Due to

* Corresponding author. E-mail: imamhuq@hotmail.com

more number of flowers it increases the yields by considerable ratio with better quality. It is specially recommended for crops like cotton, vegetable crops like chilies, brinjal, tomato and lady's finger and flowering plants such as rose, crassandra, chrysanthemum etc.

Arsenic is not an essential element either for plants or for animals. As-contaminated groundwater used for irrigation leaves a risk of accumulation of this toxic element in soil and the eventual exposure of the food chain through plant uptake and animal consumption (Imamul Huq and Naidu 2005). However, the impact of As on the growth and quality of tomato in presence of plant regulatory hormone like nitrobenzene is not yet known. As nitrobenzene is being used in tomato culture, it was thought pertinent to see the effect of this compound on the growth, yield and uptake of As by tomato plants.

Materials and Methods

Collection and processing of soil samples

The sampling site selected for collection of the soils is an adjacent area of Faridpur Sadar which belongs to Ishurdi series. The geographic location of the sampling site is $23^0$40.842′ N and $89^0$45.749′ E. The bulk of soil samples representing 0 - 15 cm depth from the surface were collected by composite soil sampling method as suggested by the Soil Survey Staff of the USDA (1951). The samples were collected with spade and dried in air for 3 days (40°C) by spreading in a thin layer on a clean piece of paper. Visible roots and debris were removed from the soil sample. After air drying, a portion of the larger and massive aggregates were broken by gently crushing them with a wooden hammer. Ground samples were screened to pass through a 2 mm stainless steel sieve. The sieved samples were then mixed thoroughly for making a composite sample. These soil samples were used for various physical analyses.

A portion of soil samples (2 mm sieved) was further ground and screened to pass through a 0.5 mm sieve. The sieved samples were mixed thoroughly for making composite samples. These soils were used for chemical and physico-chemical analyses. The rest of the soil samples collected for pot experiment was air dried, cleared off the debris and crushed to smaller clods and passed through a 5 mm sieve.

Analyses of soil samples

Various physico-chemical properties of soils were analyzed in the laboratory. Soil pH was measured electrochemically using a glass electrode pH meter at a soil : water ratio of 1 : 2.5. Particle size analysis was done by Hydrometer method. The available and total N in soil were determined by alkali distillation either of the Kjeldahl digest or of the extract for available-N. The available P of soil was determined spectrophotometrically at a wavelength of 880 nm by developing blue color using ascorbic acid after extracting the soil with 0.5M $NaHCO_3$. The available K of the soils was determined by flame photometer after extracting the soils with 1N ammonium acetate at pH 7. For the determination of total P, K and S in soil, the samples were digested with aqua-regia (HCl: HNO_3 = 3:1). The total P content in the digest was determined colorimetrically in a spectrophotometer at 490 nm by developing yellow color with vanadomolybdate. Total S content was determined by turbidimetric method. The total K in the digest was determined by flame photometer. The procudures for the determination of pH, per cent moisture, particle size, available and total N, P, K and S were followed as outlined in Imamul Huq and Alam (2005). The determination of soil As was done by Hydride generation-Atomic absorption spectrometer (HG-AAS) after digesting the sample using aqua-regia (HCl : HNO_3 = 3 : 1) (Portman and Riley 1964). Total lead, cadmium, iron, manganese and zinc content of the background soil samples were determined by Atomic absorption spectrometer (AAS) following digestion with aqua-regia (HCl : HNO_3 = 3 : 1).

Experimental Set-up

For the pot culture experiment, seedlings of hybrid variety (BARI-3) tomato (*Solanum lycopersicum*) were planted in the net-house of the Department of Soil, Water and Environment, University of Dhaka.

The treatments in the experiment were designed in two sets - soil with no arsenic treatment and soil with arsenic treatment. Recommended doses of fertilizers (NPK) were applied at three different rates *viz.*, ½ of recommended dose, ¾ recommended dose and full recommended dose. Nitrobenzene was sprayed on to the growing plants having these three different rates of fertilizer doses. Therefore, there were 14 treatments including the control treatment (7 treatments in each set). Each treatment had three replications. Accordingly, there were 42 pots: 3 pots for each treatment; 21 pots for each set. The treatment combinations were as follows:

- Control (C): No fertilizer and no nitrobenzene was applied.
- Nitrobenzene (N): Only nitrobenzene was applied.
- ½ dose of recommended fertilizer (½ RD).
- ½ dose of recommended fertilizer plus nitrobenzene (½ RD+N).
- ¾ dose of recommended fertilizer (¾ RD).
- ¾ dose of recommended fertilizer plus nitrobenzene (¾ RD+N).
- Full dose of recommended fertilizer (FRD).

Spiking of the soil was done with arsenic (As) in solution at a rate of 20 kg/ha. Na-arsenate was used as a source of arsenic. A solution was made by calculated amount of As and 100 ml of solution was added to each pot of the sets having As treatment.

Nitrobenzene solution was prepared by dissolving 10 ml of liquid in 1000 ml of water. Fertilizer was used according to BARC (2005) guideline. Nitrobenzene solution was sprayed at 10, 15, 25, 30, 40 and 50^{th} day of plant growth.

Processing of the plant samples

Tomato plants were harvested at 100 days after planting. The plants were harvested by uprooting. The harvested roots were washed with deionized distilled water several times to remove ions from the ion free space as well as to dislodge any adhering particles on the root surface. The upper parts of plants were also washed. The plant samples were separated into two parts : aerial and underground. The fresh weights of the collected plant samples were taken, air dried and then oven dried at 70±5°C for 48 hours and the dry weight of the plant sample was noted. After grounding and sieving at 0.2 mm sieve the dried samples were preserved in small plastic bottles separately.

Analyses of plant samples

Plant sample (0.5 g of both aerial and underground parts) was taken separately into 100 ml Pyrex glass tubes. Five ml of HNO_3 was added and the tubes were left for half an hour. Then the sample tubes were placed on a digestion block. Samples were normally predigested overnight over 50 - 70°C before increasing the temperature to 140°C for the final dissolution of organic material. When dissolution was complete, samples were diluted to 25 ml, shaken and filtered. This extract was used for the determination of P, K, S (Imamul Huq and Alam 2005) and As (Portman and Riley 1964) contents of the plant samples. The total N in plant samples was determined by Kjeldahl's method following H_2SO_4 digestion (Imamul Huq and Alam 2005). All elements were calculated on oven dry basis.

Statistical analyses of data

Data were statistically analyzed by using Microsoft Excel and/or SPSS (version 16) Packages.

Results and Discussion

Initial characteristics of soil

The collected soil samples were analyzed in the laboratory before setting up of the experiment to assess the nutrient status of the soil. The soil was almost neutral in reaction (pH 7.1), clayey in texture. Total As content of the soil was not high. The results of the analyses are presented in Table 1.

Table 1. The physical, chemical and physico-chemical properties of the soil.

Soil properties	Ishurdi soil
pH	7.1
% sand	15.12
% silt	37.13
% clay	47.75
Textural class	Clay
Available N (%)	0.007
Available phosphorus (mg/kg)	5.51
Available potassium (mg/kg)	17.41
Total nitrogen (%)	0.12
Total lead (mg/kg)	34.93
Total cadmium (mg/kg)	0.85
Total zinc (mg/kg)	104.75
Total manganese (mg/kg)	299.60
Total iron (%)	3.56
Total arsenic (mg/kg)	3.36

Plant height and fresh matter production

The height of tomato plants and fresh matter production were observed under two conditions *viz.* with and without As treatment. Observation was made on the aerial part and the root. The height of tomato plants and fresh, dry matter production are presented in Tables 2 and 3, respectively.

Effect of NPK and the nitrobenzene application on shoot and root length of tomato plants under both the soil conditions was better. The maximum shoot and root lengths (124.46 and 13.34 cm, respectively) in soil having no As were recorded for ½ RD+N and ¾ RD+N treatments. On the other hand, the treatment ¾ RD+N gave the highest shoot (126.37 cm) and root length (15.88 cm) in As treated soil.

Table 2. Height (cm) and fresh weight (g/pot) of tomato plants grown in arsenic free and arsenic treated Soils.

Treatments	Soil without arsenic			Soil treated with As		
	Shoot height	Root length	Total fresh weight	Shoot height	Root length	Total fresh weight
C	80.65^{d}	8.26^{b}	22.86^{d}	78.74^{f}	10.16^{b}	24.55^{f}
N	66.04^{e}	11.43^{a}	16.18^{e}	101.60^{d}	12.40^{b}	32.90^{e}
½ RD	109.22^{c}	12.70^{a}	35.57^{c}	108.59^{c}	13.34^{ab}	38.97^{d}
½ RD+N	124.46^{a}	12.70^{a}	41.75^{b}	116.84^{b}	15.24^{a}	42.75^{cd}
¾ RD	105.41^{c}	11.43^{a}	38.8^{bc}	86.36^{e}	15.24^{a}	45.55^{bc}
¾ RD+N	121.29^{a}	13.34^{a}	60.15^{a}	126.37^{a}	15.88^{a}	50.31^{a}
FRD	114.30^{b}	12.70^{a}	57.3^{a}	121.29^{ab}	13.34^{ab}	47.60^{ab}

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Fresh matter production was also increased by application of both NPK and nitrobenzene (Table 2). The highest fresh matter weight of 60.15 and 50.31 g/pot under no As and with As, respectively were noted for the ¾ RD+N treatment. Plants grown under control condition showed some symptoms like reduced plant and root growth, yellowing and wilting of leaves. In contrast, plants grown under nitrobenzene was fresh and succulent. Similar findings were found by Aziz and Miah (2009) with rice where application of nitrobenzene significantly increased the plant height, tiller and panicle production and yield of rice shoot and grain.

Nitrogen content in the plants

The mean contents of N in the aerial part of tomato plant grown under different treatments are presented in the Table 3. In case of arsenic non treated soils, the mean content of N in the aerial part varied from 0.97 to 2.62% whereas it was from 0.99 to 2.32% in case of arsenic treated soils. The accumulation of nitrogen was higher in plants grown with fertilizer plus nitrobenzene than that grown with fertilizer only. The highest content of nitrogen was found when ¾ recommended fertilizer was applied with nitrobenzene (¾ RD+N) both for As treated (2.621%) and As non treated (2.315%) soils. Rokhzadi and Toashih (2011) observed significant increase in the uptake of nitrogen of chickpea by the inoculation of *Rhizobacteria,* a plant growth promoter.

Table 3. Content of nitrogen (%) in shoot (soil without As and soil with As).

Treatments	Soil not treated with As	Soil treated with As
C	0.972[e]	0.994[e]
N	1.278[d]	1.54[cd]
½ RD	1.005[e]	1.387[d]
½ RD+N	2.042[b]	1.736[bc]
¾ RD	1.78[c]	1.747[bc]
¾ RD+N	2.621[a]	2.315[a]
FRD	2.075[b]	1.835[b]

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Phosphorous content in the plants

The mean content of P is shown in the Table 4. A higher accumulation of P was found when soil was treated with different doses of fertilizers plus nitrobenzene than that under control. In case of As free treatment, the accumulation of P in aerial part was almost same (1.22%) for ¾ RD plus nitrobenzene and FRD. However, the higher accumulation of P in root was noticed in ¾ RD plus nitrobenzene. In case of As treated soil, the mean content of P was almost similar in the aerial part of plant (0.06%) for all the treatments. The highest accumulation of P in root was observed for the ¾ RD treatment (0.737%) and lowest for the ½ RD plus nitrobenzene application (0.077%).

Table 4. Content of P (%) in shoot and root of arsenic free and arsenic treated plant samples.

Treatments	Soil not treated with As		Soil treated with As	
	Shoot	Root	Shoot	Root
C	0.060[b]	0.173[ab]	0.061	0.118[e]
N	0.060[b]	0.175[ab]	0.06	0.141[e]
½ RD	0.123[b]	0.192[a]	0.06	0.423[c]
½ RD+N	0.123[b]	0.212[a]	0.06	0.077[e]
¾ RD	1.220[a]	0.130[b]	0.06	0.737[a]
¾ RD+N	1.220[a]	0.228[a]	0.06	0.268[d]
FRD	1.220[a]	0.198[a]	0.061	0.610[b]

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Potassium content in the plants

The mean content of K is shown in the Table 5. In case of As free treatments, the accumulation of K ranged from 1.59% (C) to 2.79% (¾ RD+N) in shoot and 1.77% (C) to 2.79% (¾ RD+N) in root. In case of As treated soil, the mean content of K ranged from 1.177% (C) to 1.6% (¾ RD+N) in aerial part of plant and 1.142% (C) to 6.270% (¾ RD+N) in root. The total accumulation of K was found to be the maximum for the ¾ RD along with nitrobenzene under both As free and As treated conditions. Similar finding was noted by Erturk *et al.* (2011) who observed significant increase in the uptake of K by a plant growth promoter *Rhizobacteria*.

Table 5. Content of K (%) in shoot and root of arsenic free and arsenic treated plant samples.

Treatments	Soil not treated with As		Soil treated with As	
	Shoot	Root	Shoot	Root
C	1.590[d]	1.769[d]	1.177[c]	1.142[f]
N	1.870[c]	2.290[c]	1.600[a]	2.237[c]
½ RD	2.530[b]	2.550[ab]	1.310[bc]	1.144[f]
½ RD+N	2.620[ab]	2.470[bc]	1.310[bc]	2.010[d]
¾ RD	2.640[ab]	2.490[bc]	1.430[ab]	1.630[e]
¾ RD+N	2.790[a]	2.790[a]	1.600[a]	6.270[a]
FRD	2.730[a]	2.550[ab]	1.600[a]	3.720[b]

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Sulfur content in the plants

In case of As free soils, the accumulation of S in shoot was found to be the highest for ½ RD+N, whereas the highest accumulation in root was found for the FRD (Table 6). The mean content of S in the aerial part varied from 0.043 to 0.129% whereas it was from 0.101 to 0.227% in case of root. The content of S in shoot of As treated plant samples was almost similar (0.064%). However, the highest content in root was found for the ¾ RD+N (0.289mg/kg) and lowest for the FRD (0.064 mg/kg) treatments, respectively.

The highest content of S in FRD and recommended dose plus nitrobenzene may be due to the increased uptake of nitrogen. Higher N accumulation needs greater protein formation, and S as a constituent of a number of essential amino acids, are also accumulated at an expedited rate. Rahman *et al.* (2011) observed a highly significant positive linear relationship among elemental S, N and P uptake. They found that the uptake of S by maize significantly increased with the application of N.

Table 6. Content of S (%) in shoot and root of arsenic free and arsenic treated plant samples.

Treatments	Soil not treated with As		Soil treated with As	
	Shoot	Root	Shoot	Root
C	0.043[b]	0.101[c]	0.064	0.128[d]
N	0.064[b]	0.187[ab]	0.065	0.152[c]
½ RD	0.128[a]	0.139[bc]	0.064	0.353[a]
½ RD+N	0.129[a]	0.129[bc]	0.064	0.082[e]
¾ RD	0.064[b]	0.213[a]	0.064	0.187[c]
¾ RD+N	0.128[a]	0.205[a]	0.064	0.289[b]
FRD	0.128[a]	0.227[a]	0.064	0.064[e]

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Accumulation of arsenic in the plants

The mean content of As in the aerial part and root are presented in the Table 7. In case of As free soils, the mean content of As varied from 0.048 (C) to 0.850 mg/kg (FRD) and 0.139 (C) to 1.880 mg/kg (¾ RD+N) in aerial part and root respectively. In case of As treated soils, the maximum accumulation of As in both shoot (1.773 mg/kg) and root (11.630 mg/kg) was observed for the FRD treatment. The reasons behind accumulation of As in shoot and root of both As free and As treated plant samples could be due to vigorous growth of plants due to fertilizer application that helped in the accumulation of As along with other essential elements.

Table 7. Content of As (mg/kg) in shoot and root of arsenic free and arsenic treated plant samples.

Treatment	Soil not treated with As		Soil treated with As	
	Shoot	Root	Shoot	Root
C	0.048[f]	0.139[f]	0.282[e]	2.554[g]
N	0.171[e]	0.512[e]	0.788[c]	2.823[f]
½ RD	0.369[d]	0.701[d]	0.249[f]	3.888[e]
½ RD+N	0.508[c]	1.686[b]	0.444[d]	4.765[d]
¾ RD	0.779[b]	1.040[c]	0.444[d]	8.349[c]
¾ RD+N	0.847[a]	1.880[a]	0.929[b]	11.088[b]
FRD	0.850[a]	1.750[b]	1.773[a]	11.630[a]

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Conclusion

From the experiment, it was observed that the growth of plants was comparatively better in soils where three-fourth of recommended fertilizer along with nitrobenzene were applied. The concentration of essential elements was also found higher in plants grown in soils where three-fourth of the recommended fertilizer and nitrobenzene were used compared to other treatments in spite of a few exceptions. So, we can use nitrobenzene along with a portion of recommended fertilizer dose. This will reduce the pressure on use of fertilizer. Compared to FRD, the treatment of nitrobenzene could alleviate As accumulation in tomato plants. This could be another mitigation approach to combat arsenic toxicity in food chain. The research on the effect of nitrobenzene on plant growth and nutrient content is very limited. More research is needed to be carried out to know whether nitrobenzene can be used as a plant growth promoter or not.

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