Original Research Article

LABORATORY GERMINATION EXPERIMENT FOR ASSESSING THE PHYTOTOXIC EFFECT OF ARSENIC ON THE GROWTH ATTRIBUTE OF DIFFERENT CROPS

ABSTRACT

Arsenic (As) pollution is a major environmental and agricultural issue across the world. Due to industrialization and over exploitation of ground water are the two most important factors for the occurrence of arsenic in the water. The irrigation of arsenic contaminated water poses threat not only to the plant system ultimately it affects all the living forms on the global. Early germination of seedlings is affected by the concurrent increase in As exposure in agricultural fields. The arsenic in very low levels also brings damage to the plant system phenotypically and metabolically. This study was carried out to evaluate the germination performance, initial growth characteristics and chlorophyll content of different crops *viz.,* rice, maize, black gram, groundnut and tomato in response to the different level of As concentration (0.1, 0.2, 0.4, 0.6, 0.8, 1, 10, 20, 40 and 60 mgL^{-1}) in the growing solution. Growth metrics in all crops were reduced as As content was increased. Among these crops rice was most tolerant to the level of 60 $mgL^{-1}$of As concentration compared to other crops. The current study's findings revealed that tomato was most susceptible to As concentrations, whereas rice was the most resistant to As stress.

Keywords: Arsenic, phytotoxicity, germination percentage, growth attributes, dry matter production, chlorophyll content.

1. INTRODUCTION

Arsenic (As) is a metalloid compound found in both organic and inorganic forms. Arsenic has been designated as a group 1 element in the carcinogenic categorization [1]. The natural or geogensic emergence of arsenic is a worldwide issue with a variety of health repercussions on humans and wildlife. As occur in organic and inorganic forms and with Inorganic As^{3+} being more mobile and poisonous than Inorganic As5+ species.

Arsenic-polluted water supplies, soils and sediments were major causes of food chain contamination and drinking water in several nations, including India (West Bengal, Assam, Bihar, Uttar Pradesh), Nepal, Germany, Pakistan, China, Bangladesh, Japan, Thailand, Vietnam and the United States etc. The guideline for As in drinking water is 10 µg/L [2], and the permissible limit of As for irrigation water is 100 µg/L [3].

Arsenic can be absorbed by plants, especially farm crops like cereals, vegetables and fruits once it has been released into the soil and humans can be harmed through consuming these polluted agricultural goods. When arsenic is present in irrigation water or in soil at high rates, it induces toxicity symptoms such as retarded plant height, decline in shoot and root growth, reduction in biomass, reduction in photosynthesis, and yield loss [4].

Arsenic accumulates in plant parts in the following order: roots >stem > leaves > edible portions [5]

The seed germination is the first physiological process which is influenced by metals stress [6]. Seed is one of the most important component in the agricultural production system The stages of seed germination and seedling development are crucial in the plant life cycle [7], as a larger number of healthy seedlings ensures a larger crop stand in the field and subsequently higher yield.

In the present study, arsenic-induced toxicity in different types of crops like rice, maize, black gram, groundnut, tomato was studied. The intention of the study was to examine the seed germination, morphology of seedling growth, dry matter production and chlorophyll content which are the most affected characteristics by metal exposure.

2. MATERIAL AND METHODS

2.1 Laboratory assay for seed germination

This study employed certified seeds of five different crops *viz.,* maize (CO 13) (*Zea mays*), tomato PKM 1 (*Solanum lycopersicum*), groundnut CO6 (*Arachis hypogaea*), rice CO 51 (*Oryza sativa*) and black gram CO 6 (*Vigna mungo*).

Prior to germination, the seeds were surface sterilized in various concentration of sodium hypochlorite such as 4% sodium hypochlorite (rice, maize, blackgram) and 2% sodium hypochlorite (Tomato, groundnut) then rinsed with distilled water.

Sodium arsenate ($Na_2HAsO_4.7H_2O$) has been used to make arsenic solutions with different concentrations of Control (Distilled water), 0.1, 0.2, 0.4, 0.6, 0.8, 1, 10, 20, 40 and 60 mg L^{-1}. To assess seed germination, a germination sheet was utilized in a modified roll towel method [8]. Each germination sheet contained twenty five seeds of each crop variety and they were kept at 23°C in the dark with a plastic sheet over it. Three replications of each treatment were performed. The rolls were then dipped in different concentration of arsenic solution along with control where the towel was kept in distilled water. Germination percentage was counted in seven days for black gram and maize, ten days for groundnut, and fourteen days for tomato and rice.
Germination and growth measurements

Germination percentage (%) $= \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown}$ x 100

2.2 Morphological Studies

2.2.1 Root length

Root length of seedlings was measured from the stem base to the longest root tip and expressed as cm.

2.2.2 Shoot length

Seedlings were randomly selected from each replication and shoot length was measured from the collar area to the longest leaf tip and quantified in cm.

2.2.3 Vigour Index

Seedling vigour index (SVI) was calculated using seedling length (SL) as follows [9]:

SVI = Seedling Length (cm) × Germination Percentage (%)

2.2.4 Dry matter production

The seedlings used for growth measurement were placed cover and dried under shade for 24 h and then kept in an oven maintained at 85±2°C for 24h. The dried seedlings were removed from the hot air oven and cooled in the desiccators over silica gel. Dry weight was recorded and the mean values were expressed in g 10 seedlings-1.

2.3 Chlorophyll content

Chlorophyll pigment was measured at 663 and 645 nm and expressed as mg g^{-1} using the DMSO method [10].

2.4 Statistical Analysis

The experiment was carried out in a completely randomized design (CRD) with three replications, each germination sheet containing an average of twenty-five seeds. ANOVA table was used to statistically assess all of the experiments. The critical difference was worked out at 5 percent (0.05) probability or corrections among parameters were at 95 % of significant level.

3. RESULTS AND DISCUSSION

The effect of arsenic on germination percentage, seedling length, vigour index, dry matter production and chlorophyll content in different crops was investigated.

3.1. Germination percentage

Seed germination is one of the most vulnerable processes in the metal polluted environment and hence this is an important consideration while studying effects of heavy metals on seedling growth. Germination percentage was significantly declined with increasing concentration of arsenic among all the crops (Fig.1). The germination percentage in black gram, maize, rice, tomato and groundnut was observed to be 92%, 92%, 96%, 88% and 88% in control and declined to 8%, 4%, 60%, 4% and 4% at 40 mg L^{-1} of arsenic in all the crops respectively. The arsenic concentration at 60 mg L^{-1} rice crop alone showed the 56% of germination no other crop was germinated at that concentration. Among the crop studied rice was observed to more tolerant and tomato was the most sensitive to the higher level of arsenic concentration. Srivastava *et al.,*(2013) reported that the *Vigna mungo*, 97 percent germination was observed in the control treatment, but 83 and 70 percent in the 100 and 200 µM As treatments, respectively [4].

Singh *et al.,*(2016) tested four different varieties (Richharia, Rajendra suvasini, Swarna-Sub 1 and Rajendra masuri) of rice in various concentration of As *viz.,*10, 20 and 30 mg L^{-1}. As the concentration of arsenic increased, the percentage of germination, the germination index, and the relative germination rate all dramatically decreased compared to the control. At a dosage of 30 mgL^{-1}, none of the cultivars experienced germination. In Richharia, the arsenic-induced damage was greater. At 10 mg L^{-1}, Rajendra suvasini, Swarna-Sub 1, and Rajendra masuri varieties reported reduction in germination more than 50% compared to control [11].

Bag *et al.,*(2019) shown that increasing concentration of As from 50 to 100 mg L^{-1}, decreased germination percentage about 46% and it was still curtailed more than 62% when the concentration increased from 100 to 150 mg L^{-1}. This is because of the energy for germination of seeds, growth of roots and shoots is provided by sugar metabolism and for this purpose α-amylase converts endospermic stored starch into metabolizable sugars which is not adequate in arsenic stress condition [12]. Similarly Baruah *et al.,*(2019) reported that arsenic directly influenced the reduction of seed germination, permeability behaviour of cell membrane and on the activity of enzyme such as amylase and protease causing inhibition of food supply to the growing radicle and plumule [13].

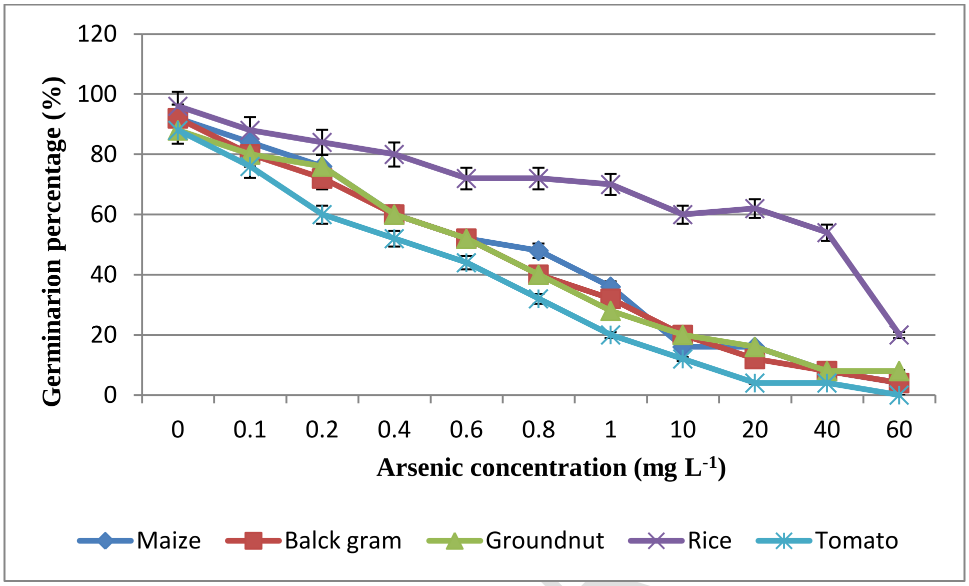

Fig.1. Effect of different concentration of arsenic on Germination percentage of crops. Each data point is the mean of three replicates. Error bars represents ±SE.

3.2. Root and Shoot length

Exposure of crops to various concentration of As significantly affected the shoot and root characteristics were presented in the (Fig. 2, 3, 4, 5, 6). Shoot and Root length decreased significantly with the increase of arsenic concentrations. Analysis of variance showed that root and shoot length in arsenic concentrations had significant effects (p=0.05).

The root length was observed to be 10.11±0.02, 9.56±0.33, 11.81±0.16, 5.71±0.81 and 7.17±0.06 cm in black gram, maize, rice, tomato and groundnut respectively in control. With the increase of concentration of As all the investigated crops showed significant reduction in root growth. Rice showed better root growth compared to other studied crops at 60 mg L^{-1} of As.

Similarly the shoot length of the crops was also studied to assess the impact of As. The shoot length was 9.23±0.04, 11.64±0.18, 9.42±0.41, 14.65±0.97, 4.21±1.16 cm in maize, black gram, groundnut, rice and tomato respectively in control (distilled water alone).Whereas the shoot length was decreased with increasing concentration of As. Rice showed better shoot growth compared to other studied crops at 60 mg L^{-1} of As. The root length might have a relatively strong suppressive effect than that of the shoot length. Biswas *et al*.,(2016) reported that trivalent (As III) and pentavalent (As V) arsenic are accumulated by plants. These forms interfere with phosphate in various metabolic pathways, and interact with sulfhydryl groups on proteins. Due to their initial contact with arsenic compounds, roots have been shown to be more influenced than shoots [14]. Similarly, Bianucci *et al*.,(2017) investigated the influence of As on peanut plants reduction in plant growth, as well as a loss in root volume, architecture, and lateral root development [15].

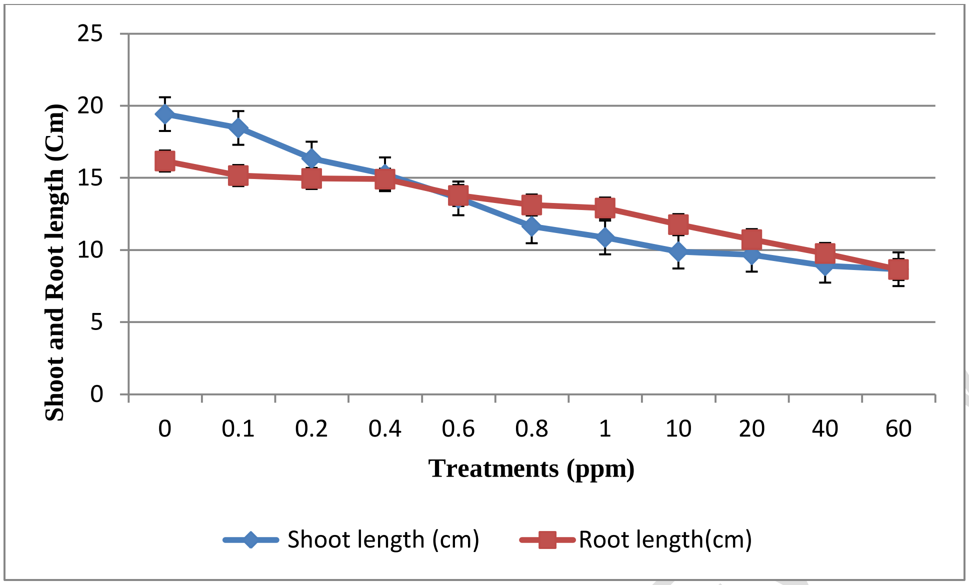

Fig.2. Effect of arsenic on the root and shoot growth of groundnut. Each data point is the mean of three replicates. Error bars represents ±SE.

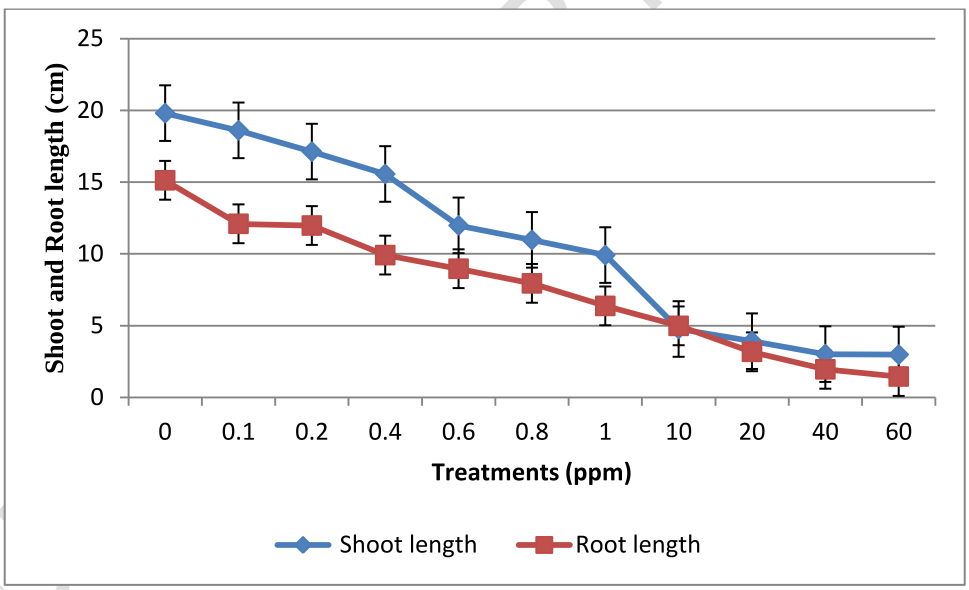

Fig.3. Effect of arsenic on the root and shoot growth of rice. Each data point is the mean of three replicates. Error bars represents ±SE.

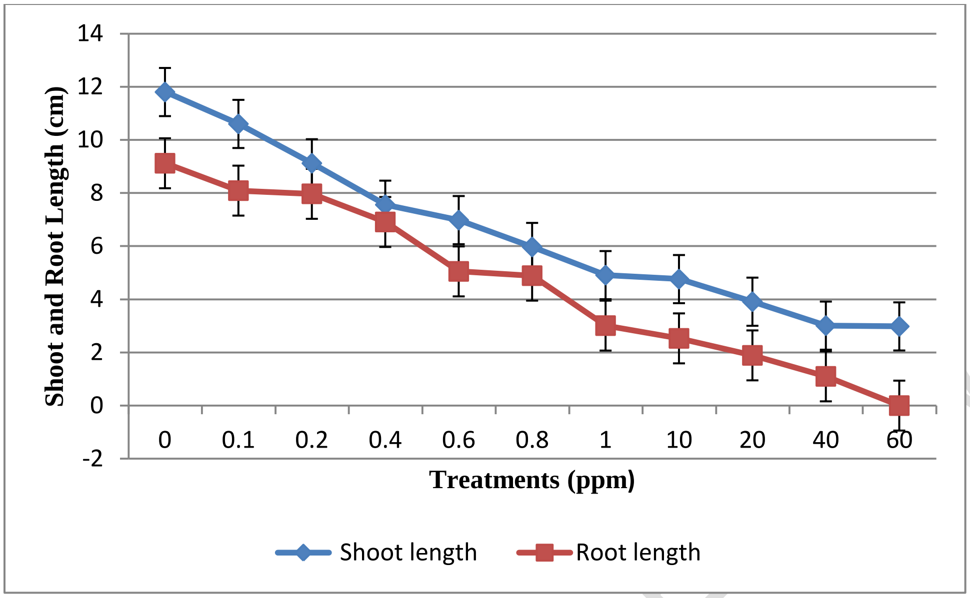

Fig.4. Effect of arsenic on the root and shoot growth of black gram. Each data point is the mean of three replicates. Error bars represents ±SE.

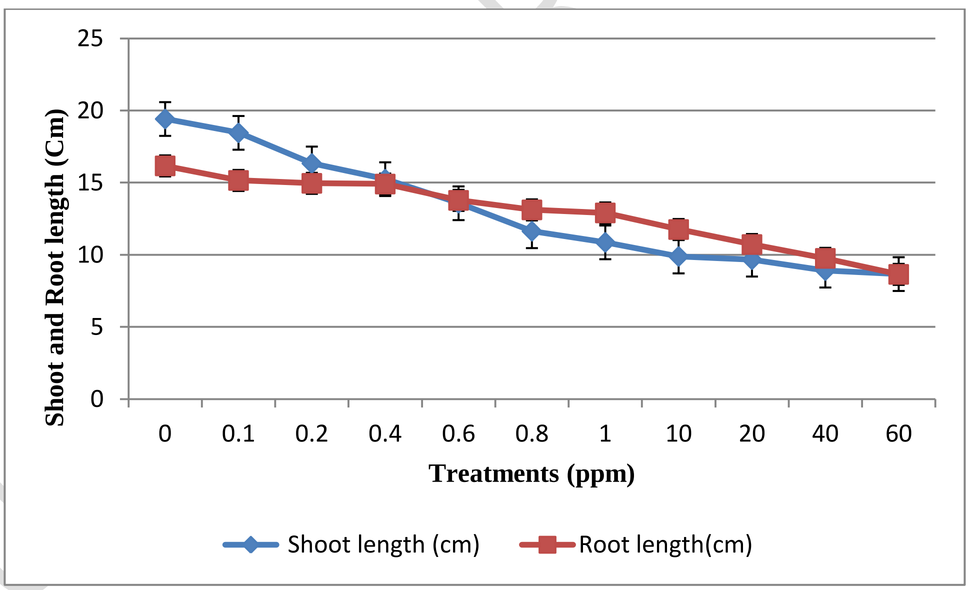

Fig.5. Effect of arsenic on the root and shoot growth of maize. Each data point is the mean of three replicates. Error bars represents ±SE.

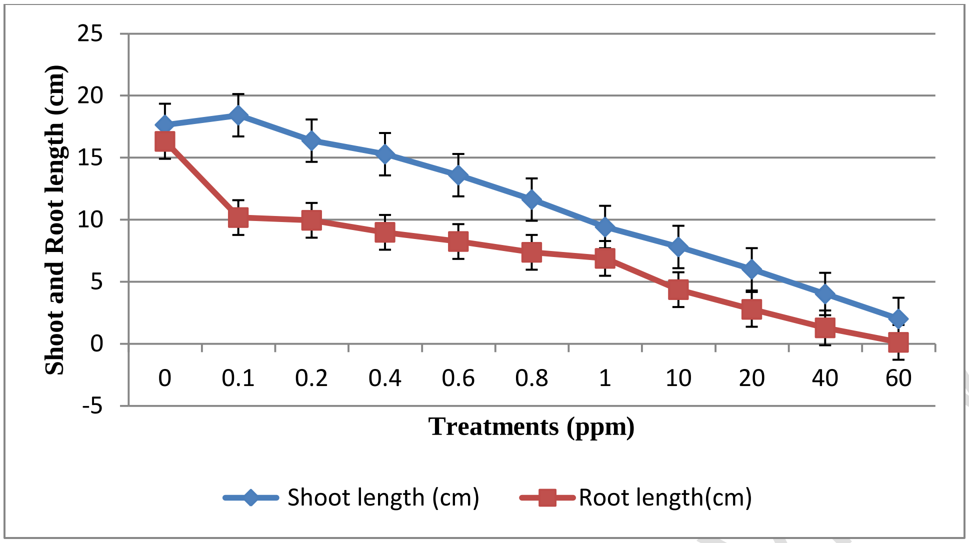

Fig.6. Effect of arsenic on the root and shoot length of tomato. Each data point is the mean of three replicates. Error bars represents ±SE.

3.3 Seedling vigour index

The seedling vigour index of different crops revealed that the As treatments had a significant impact on seedlings. Analysis of variance showed that seedling vigour index was significantly effects (p=0.05). In black gram, maize, rice, tomato and groundnut, the seedling vigour index was observed to be 1288, 2925, 3123, 873 and 1874in control and declined to 42, 88, 512, 16 and 29at increased level of arsenic in all the crops at 40 mg L^{-1}. Among all these crops, rice has highest level of vigour index (3123) at all concentration (Fig.7). Biswas *et al.*,(2016) reported that the vigour index exhibits a direct decrease when the concentration of sodium arsenate is increased since it depends on root length, shoot length, and germination percentage[14].

This result was in line with study conducted by Baruah *et al*.,(2019), the results of the experiment revealed that the seedlings treated with different concentration of different heavy metals showed the highest reduction (83.4%) in vigour index in 175 mg $L^{-1}$of copper (Cu) in wheat followed by 220 mg L^{-1} of lead (Pb) in pea (79.2%) and 220 mg L^{-1} (Cd) of cadmium in wheat(78.8%)[13].

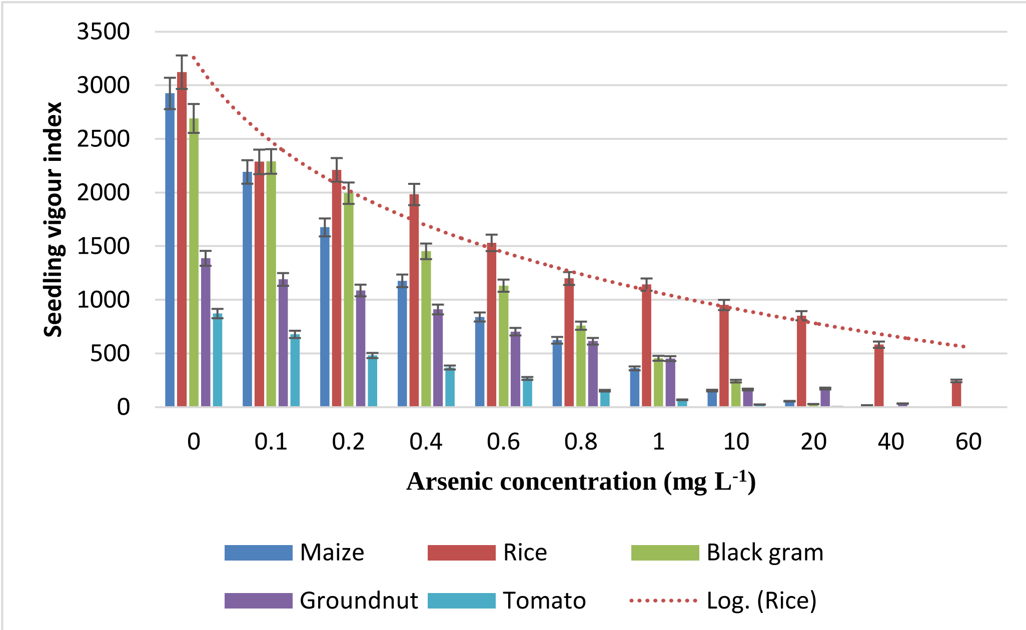

Fig.7. Effect of arsenic on the seedling vigour index of different crops. Linear line indicates that highest vigour index on rice in among all the crops.

3.4 Dry matter production

Seedling biomass is diminished as arsenic levels have increased a notable reduction in dry matter production in all the crops except in rice crop was observed (Fig.8). In The results obtained for root and shoot length of seedlings was linear to biomass production of the crops. Analysis of variance showed that dry matter production was significantly effects ($p=0.05$). Similar study was conducted by Ghosh *et al.,*(2016) that the influence of As (v) on maize seedlings at 15 and 30 mg L^{-1}.They noticed a considerable reduction in dry matter production of seedlings, as well as yellowing and drying of the leaves, as well as a lack of viable root growth[16].

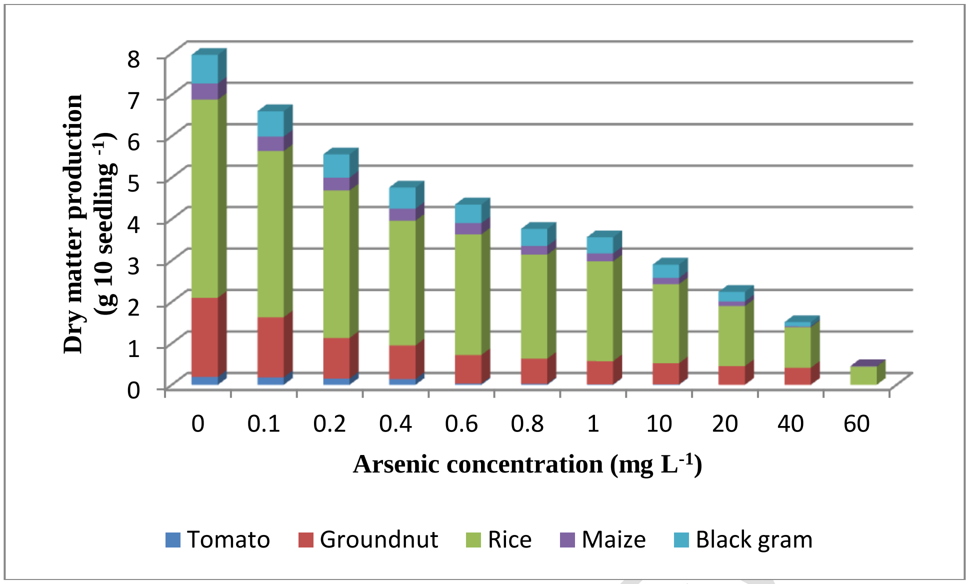

Fig.8. Effect of arsenic on the dry matter production of different crops.

3.5 Chlorophyll content

Chlorophyll content was significantly decreased among all crops with increasing level of As as represented in (Fig.9). Analysis of variance had significantly effects chlorophyll content (p=0.05). The reduction may be due to the disruption of photosynthetic pigments by arsenic in black gram, rice, maize, groundnut and tomato. In control treatment the chlorophyll content was0.65, 4.62, 0.45, 3.96, and0.36mg g^{-1} of in black gram, rice, maize, groundnut and tomato respectively. The chlorophyll content was decreased to 0.1 mg g^{-1}in maize and 2.11 mg g^{-1} in rice at 60 mg g^{-1}concentration of arsenic. The decline of adaptive changes of pigment synthesis to high arsenic levels is indicated by the significant decline in pigment content in arsenic-treated seedlings. Among all these crops rice was tolerance to higher concentration of As (60 mgL^{-1}) (Fig.9). Srivastava *et al*.,(2013) reported that *Hydrilla verticillata* plants in chlorophyll a and chlorophyll b content decreased similarly at greater dosages of arsenic treatment and biosynthetic route in decrease in chlorophyll synthesis caused by the production of ROS Upadhyaya *et al*.,(2014). According to[14] higher arsenic concentrations can cause the damage to the shape of the chloroplast which changes in the accumulation of chlorophyll contents in rice leaves can also result from higher arsenic concentrations. Baruah *et al.*,(2019) reported that the reduced carbohydrate content of the emerging seedlings under metal exposure might be due to inhibition of chlorophyll biosynthesis as observed from the lower chlorophyll content[13].

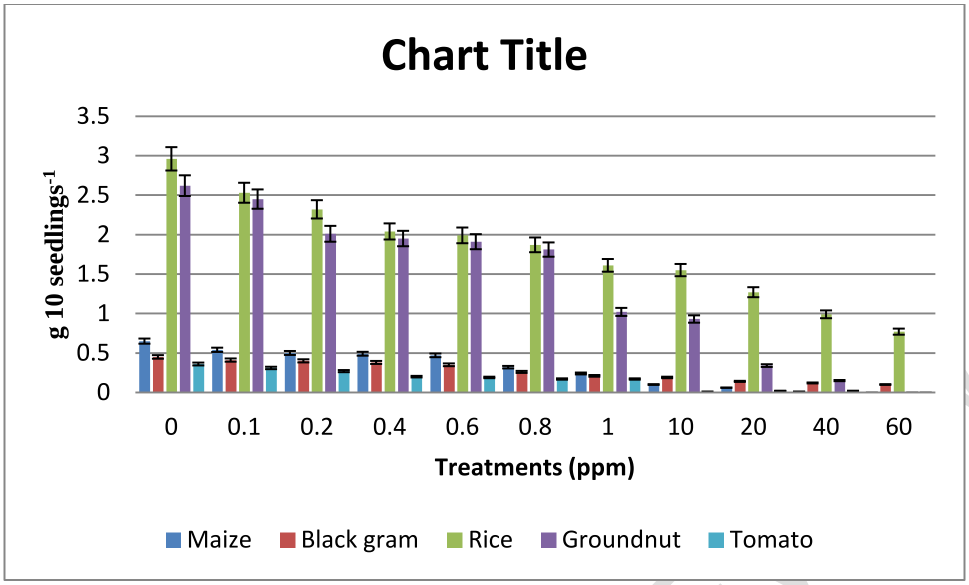

Fig.9. Influence of arsenic in chlorophyll content of different crops. Each data point is the mean of three replicates. Error bars represents ±SE.

4. CONCLUSION

This study shows that As was known to inhibit the processes of seed germination, root and shoot growth, and other earlier developmental processes that occur during the early stages of seedling development at 40 mg L^{-1}. At elevated concentrations of arsenic, overall reduction in vegetative response viz., germination percentage, shoot and root length, seedling vigour index, dry matter production and chlorophyll was observed. Root growth in seedlings was significantly affected than shoot growth. On comparing the effects exhibited by different crops at various concentrations of arsenic, rice was identified to have the highest tolerance at 60 mg L^{-1} and tomato is observed to be sensitive at 1 mg L^{-1} for the arsenic. From this study it can be concluded that presence of arsenic in the soil in higher concentration is toxic to almost all the crops. However, knowledge of the impacts of metals on crops, particularly the causes of damage, is still not known. Futher studies on how the arsenic accumulation in plants can be undertaken to understand its impact on production crops.

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