Original Article

Journal of Plant Stress Physiology 2016, 2: 31-35
http://scienceflora.org/journals/index.php/jpsp/
doi: 10.19071/jpsp.2016.v2.3107

Evaluating the growth potential of *Vigna radiata* (green gram) using *Albizia amara* and *Leucaena leucocephala* as a phytoremediator for textile dye (Navy blue dye) simulated soil

N. Sudarmani Gayathri, V. Jayanthi*

Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Received: 22.08.2016
Accepted: 12.10.2016
Published: 17.12.2016

***Address for Correspondence:**
V. Jayanthi, Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.
Phone: +91-9894707275.
E-mail: sendanicemail3@gmail.com

ABSTRACT

Soil pollution, due to textile dye affects the soil fertility, is a cause for demand crop production in agriculture. The present investigation was to evaluate the growth level of *Vigna radiata* (green gram) before and after simulation of textile navy blue dye in soil. The growth potential of Vigna radiata (green gram)before and after remediation were assessed using seed germination. Percentage, biometric evaluation (root length, leaf area, shoot length, and plant height), and biochemical activity (Total protein, amino acids, DNA, total carbohydrate, and amylase activity) in root tissue. All the parameters carried out with maximum of 1% concentration of textile dye to assess the remediating capacity of plant biomass *Albizia amara* and *Leucaena leucocephala*. On assessing, the use of plant adsorbents *Albizia amara* and *Leucaena leucocephala* as phytoremediator has enhanced the binding capacity of blue dye to a great extent shows the phytotoxicity of dye up to 1% in soil.

KEY WORDS: Phytoremediator, *Albizia amara*, *Leucaena leucocephala*, textile dye (navy blue), germination study, biometric evaluation, biochemical estimation

INTRODUCTION

Textile dye stuffs discharge from industries during the coloration process enters water body and binds the soil increases soil contamination. Approximately, 45% dyes are released into the environment during dyeing process making the effluent highly colored and esthetically unpleasant (Wang *et al*., 2002). The dyes which have high water solubility travel toward the land through rivers and sewage lead to soil contamination and affects food chain which on further destroy the living organisms (Imen *et al*., 2010). Hence to protect the plant from potential hazard an eco-friendly technique called phytoremediation, to achieve soil stabilization, minimization of contaminant leaching and esthetic improvement at cost effect often efficient for heavy metal removal (Karimi, 2013). The two plant source leaf biomass *Albizia amara* (AA) and dry pod biomass *Leucaena leucocephala* (LL) were used as adsorbents to remove navy blue dye in simulated red soil. This study helps to remove the toxicity level in contaminated sites and to improve the plant growth.

MATERIALS AND METHODS

Textile Dye

Navy blue dye was chosen for the study since it is widely used in textile dyeing process. Navy blue dye was purchased from a whole sale supplier near to the dyeing industry in Tirupur. Range finding test was performed and observed the toxicity level of dye was up to 1% and chosen for the further study. Physiochemical properties of textile navy blue dye are characterized for their color, type is an organic dye, pH-11.6, highly soluble in water, and specific gravity of 0.835 and ultraviolet visibility around 620 mn and its melting point is above 400°C was identified and used for the assessment of phytoremediation.

Selection of Soil for the Study

Virgin red soil is suitable for all plant cultivation, maintains the moisture, and texture. Virgin red soil from Sulur, Coimbatore, was selected for the study. It was cleaned of debris and stones and used for the study. Pot study

was carried out with 1 kg of soil, 1% of dye solution, and 250 g of phytoremediator. Soil analysis indicates dye contamination can change the macronutrients such as N, P and K content, micronutrient such as copper and manganese, iron and zinc status of the soil.

Selection of Plant Material for Phytoremediation

AA and LL were selected as plant source for phytoremediation. Plant indexing for both the plant sources was done at BSI index. AA BSI/SRC/5/23/13-14/Tech-2055 and LL BSI/SRC/5/23/10-11/Tech-448. AA leaves were shade dried, powdered, and used. Dry pods of LL were powdered and used. The two forms of plant source were studied separately and in combined form in the ratio of 1:2 by weight.

Assessment of Plant for Phytoremediation

Co-6 variety of green gram (*Vigna radiata*) seeds was obtained from TNAU. The seeds were stored, uniform sized seeds were selected for the study. Green gram grows well in any soil and thus was chosen for the study. It was sterilized with 0.5% mercuric chloride and washed thrice with distilled water and air dried for a few seconds before using it for germination study.

Preliminary Phytochemical Screening of Plant Biomass

The phytochemical screening of 50% ethanolic extract of LL and AA was used and identified for further study. The result reveals the presence of Alkaloids (Waldi, 1951), flavonoids (Harborne, 1987), tannins (Evans, 1997), phenols and Saponins (Mace, 1963), carbohydrates (Ramakrishnan and Rajan, 1994), proteins (Fisher, 1968) and high intensity of flavonoids and phenols.

Seed Imbibitions Rate

Imbibition study helps to identify the germination process. Seed imbibitions rate of green gram was studied with different concentration of dye with 0.25%, 0.50%, and 1% at different time interval up to 5 h maximum. After each time interval, the seed is dried and the weight of seed is calculated before and after imbibition in dye solutions. The percentage of dry weight and wet weight was calculated using the formula (C−T/C*100) and is plotted in graph (ISTA, 1985).

Quantitative Determination of Phenols and Flavonoids

Phenols and flavonoids are important phytoconstituents in plant plays a role in physiological health and molecular mechanisms was quantified using the standard methods used by Cameron *et al*. (1943). The validation was done and reveals the presence of flavonoids and phenols in AA has 10.5 mg catechol and in LL has 9.9 mg catechol and 2.9 mg catechol.

Seed Germination

Seed germination is the physiological sense and can be considered to be complete, when embryo growth is initiated. Germination percentage of *V. radiata* (green gram) is calculated after the emergence of radical through the seed coat. Germination period was maintained up to 3 days. The number of seeds germinated was counted and calculated using the formula:

% germination=No: of seedlings/No: of seeds sown×100

Biometric Evaluation of *V. radiata* (Green Gram)

The biometric evaluation is the potential tools in monitoring the ecological stress in plants. Biometric studies of plant *V. radiata* (green gram) was done, (i) root length, (ii) shoot length, (iii) plant height, and (iv) leaf area. The measurement was précised using measuring scale, and the values were expressed in graph. Leaf area was calculated by measuring length and breadth of leaf as described by Yoshida and Tracey (1993).

Biochemical Estimation of *V. radiata* (Green Gram)

With the exception of root crops, plant root studies are limited than shoot system. The root systems were maximum accumulation and damage occurs due to dye toxicity was taken as a measure of stress. Stress influences the evolution of plant shoot, which follows after the advent of roots. Thus, soil-root studies are considered more vital and root extract was taken for the following analysis the roots taken for the analysis was from plants grown up to 17th day. The biochemical parameters total protein (Lowry, 1951), total carbohydrate (Hedge and Hofreiter (1962), amino acids (Jones and Kielland, 2012), DNA (Pikovskaya, 1948), and amylase (Bernfield (1955) on the root extract of *V. radiata* (green gram) before and after remediation were estimated.

RESULTS AND DISCUSSION

Seed Imbibition Rate

The seed imbibitions study is carried out with *V. radiata* (green gram) with a minimum of 0.25% to a maximum of 1%, concentration of dye. Rate of imbibition reveals the rate of absorption of dye at different time interval as calculated and depicted below in Figure 1.

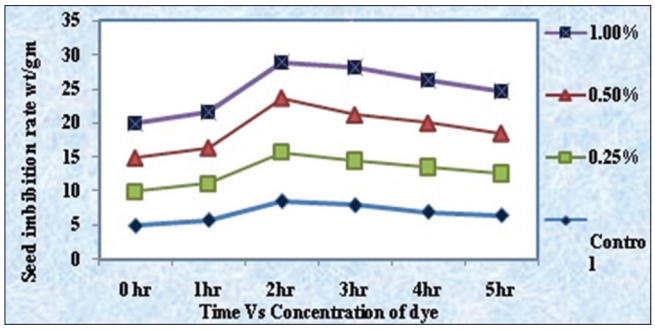

Figure 1: Inhibition study of *Vigna radiata* (green gram)

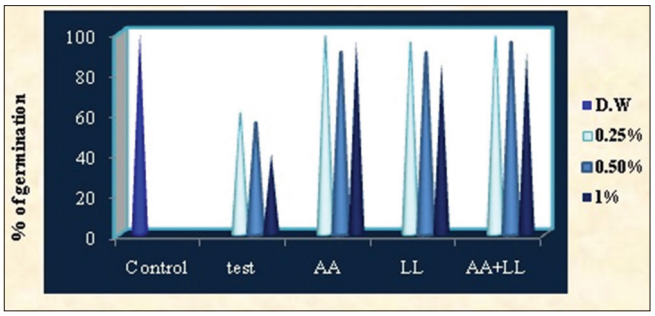

Figure 2: Germiability of *Vigna radiata* (green gram)

Maximum absorption rate of dye by *V. radiata* (green gram) seed occurs within the first 2 h after which a similar pattern of absorption occurs in control and in dye treated seeds. The rate of imbibition is influenced by the solute rather than the dye present in it. High activity of α-amylase could causes starch granule damage and changes in the physicochemical properties of starch which could be a contributory reason in germinating seeds (Wiwart *et al.*, 2006). This may suggest that at the initial stage of imbibition water penetrates very quickly into the seeds characterized by advanced starch degradation, and equally quickly gets out of them. These processes are not accompanied by changes in seed volume.

Seed Germination

Germinability of plants strongly depends on soil to germinate and grow, so any alterations in the seed development may reflect the presence of toxic substances in the soil. Hence, germination test in ecotoxicological assays is considered short-term and evaluates acute toxicity effects (Cruz *et al.*, 2013). A study on the percentage germination of *V. radiata* (green gram) on dye treated and remediated with AA, LL and AA + LL was assessed. The soil was amended with different concentration of dye (0.25%, 0.50% and 1%) was used for germination study of green gram. A reference control was maintained without the dye. The percentage germination was calculated up to the third day after sowing when the maximum activity occurs.

Figure 2 reveals the toxicity induced by dye on *V. radiata* (green gram) germination and seedling growth. The results showed that seedling growth is more sensitive to dye than seed germination. Comparing the rate of germination of control, test and remediated, control seeds had 100% germinability, whereas test had 35-60% based on the concentration of dye simulated. On remediation with AA, LL and AA + LL the percentage germinability increased above 85% in all concentrations of dye. *V. radiata* (green gram) seed with dye and remediated did not show any inhibitory effect on seed germination at minimum concentration of 0.25%. Although the seeds germinated at 1% dye, they did not survive for longer periods. The better growth was recorded at 0.25% dye concentration.

The germinability of seeds is importantly related to the agricultural scenario for the removal of dyes which consumes a large area of soil. It is to evaluate to assess the degree of toxicity since this could affect the viability of seeds.

Biometric Evaluation

Figure 3 explains the biometric evaluation of root length, shoot length, plant height, and leaf area was assessed in *V. radiata* (green gram) plant grown before and after remediation of dye simulated soil. A pot experiment is carried out with different percentage of dye from minimum concentration of 0.25%, 0.5% and maximum concentration up to 1% to categorize the growth level of (*V. radiata*) green gram using a plant biomass AA and LL.

Biometric studies explain the development status of root, shoot, plant growth, and leaf area of *V. radiata* (green gram). There was a significant change in plants grown in remediated soil compared to dye simulated soil. Thus, the study reveals that the plant biomass AA, LL and AA+LL used as phytoremediator for dye removal helps to promote plant growth in dye contaminated soil up to 1% level. Similar results are observed with, sunflower in Evans blue dye contaminated soil (Xie *et al.,* 2014).

Biochemical Analysis of *V. radiata* Root before and after Remediation

The biochemical changes observed were for protein, total carbohydrate, and DNA. Comparison between control, dye simulated and remediated roots were made.

Table 1 shows the biochemical changes observed in root tissue of *V. radiata* (green gram) grown in dye simulated, remediated and control soil. The plant roots in dye

Table 1: Comparison of biochemical changes observed between control, test and remediated roots of *V. radiata* before and after remediation

Groups	Total protein (mg/g of tissue)	Amino acids (mg/g of tissue)	DNA (mg/g of tissue)	Total carbohydrates (mg/g of tissue)	Amylase (units/g of tissue)
Control (G1)	28.3±1.18	0.16±0.01	1.91±0.11	35.1±1.41	59.7±1.19
Test (G2)	16.7±0.97*[a]	0.34±0.01*[a]	1.03±0.07*[a]	10.8±1.09*[a]	28.9±0.80*[a]
AA (G3)	38.8±1.57[a]*/[(ns)]	0.18±0.02[a]*/[(ns)]	2.78±0.09a*/[(ns)]	30.1±1.33*[a]/[(ns)]	70.8±1.29*[a]/[(ns)]
LL (G4)	36.6±1.39[a]*/[(ns)]	0.14±0.02[a]*/[(ns)]	2.55±0.08*[a]/[(ns)]	29.5±1.12*[a]/[(ns)]	61.6±1.13*/*[ab]
AA+LL (G5)	39.5±1.70*[a]/[(ns)]	0.21±0.02*[a]/*[ab]	2.99±0.12*[a]/[(ns)]	34.5±1.48[a]*/[(ns)]	69.6±1.20*[a]/[(ns)]

1 unit of amylase activity=μ moles of maltose released. Values are mean±SD, n=6, group comparison: G1 versus G2; G2 versus G3, G4, G5; G5 versus G3, G4. Statistical significance: $P<0.05$.*a: Highly significant,*ab: Significant, ns: No difference

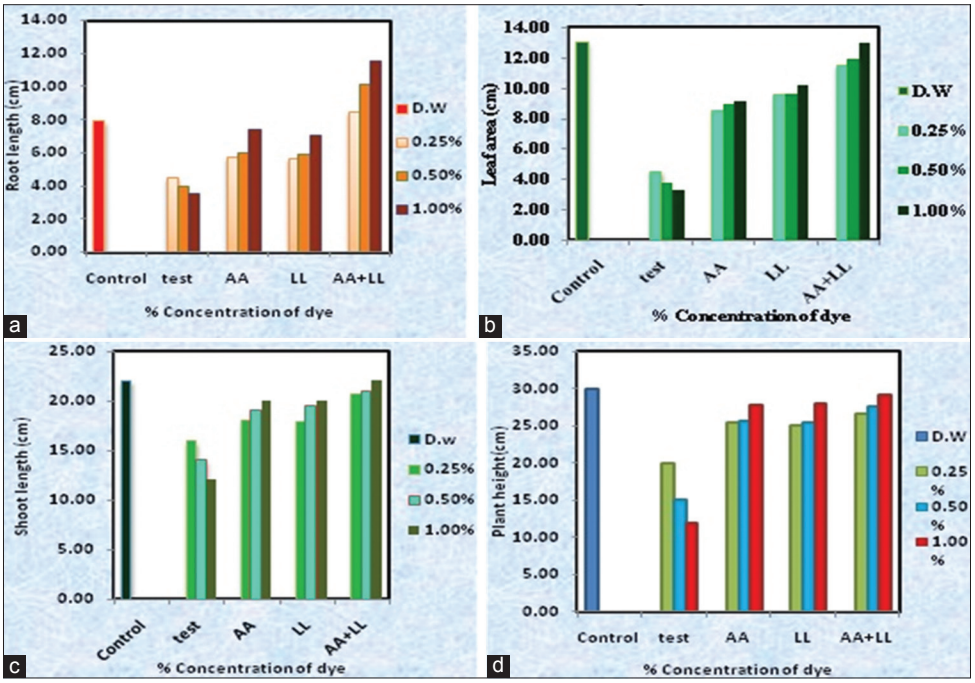

Figure 3: Biometric assessment of *Vigna radiata* (green gram). (a) Root system, (b) leaf area, (c) shoot system, (d) plant height

simulated soil shows significant decrease of protein, DNA, total carbohydrate, and amylase activity. Amino acids in dye simulated roots shows highly significant compared to that of remediated and control roots. When compared between control and remediated roots, the plant biomass AA and LL and combined plant biomass (AA + LL) used for phytoremediation enhances the root growth and improved the biochemical activity and shows significant increase in protein, DNA, carbohydrate, and amylase. Comparison within the remediator AA + LL shows high activity.

DISCUSSION

Germination study showed that the plant biomass had the potential of removing the dye toxicity and enhanced seed germination. Biometric studies revealed that the development of root, shoot, plant growth, and leaf area of *V. radiata* (green gram) was reduced in dye simulated soil and enhanced on remediation. AA, LL and AA+LL show the ability to remediate the phytotoxicity caused by navy blue dye on *V. radiata* (green gram). Biochemical estimation revealed that level of protein, DNA, total carbohydrate, and amylase was significantly high in plant biomass AA+LL with regard to AA and LL. This study showed that the plant biomass AA and LL have the potential to grow *V. radiata* in textile dye simulated soil.

CONCLUSION

From the above evaluation, it concludes that leaf biomass AA and dry pod biomass of LL was a potent phytoremediator has the remediating capacity for simulated textile navy dye and improved the growth rate of *V. radiata* (green gram).

ACKNOWLEDGMENTS

We would like to express our gratitude to the PSG College of arts and science, Coimbatore and the authorities of

Bharathiar University, Tamil Nadu, India, for their support to this research work.

REFERENCES

Bernfied P. In: Colowick S, Kalpana NO, editors. Methods of Enzymology. Vol. 1. New York: Academic Press; 1955. p. 149.

Cameron GR, Milton RF, Allen JW. Measurement of flavonoids in plant samples. Lancet 1943;179.

Cruz JM, Lopes PR, Montagnolli RN, Tamada IS, Maria N, Silva MG, *et al*. Toxicity assessment of contaminated soil using seeds as bioindicators. J Appl Biotechnol 2013;1:1.

Evans WC. An index of medicinal plants. A Text Book of Pharmacognosy. 14th ed., Vol. 7. 1997. p. 12-4. Ecobiol 19:19-22. Electrochemical methods. Water Res 28:277-82.

Fisher DD. Protein staining of ribboned upon section for light microscopy. Histochemistry 1968;16:81-96.

Harborne JB. Methods in Plant Biochemistry. London: Academic Press; 1987.

Hedge JE, Hofreiter BT. Determination of total carbohydrate by anthrone reagent. In: Whistler RL, Be Miller JN, editors. Carbohydrate Chemistry. New York: Academic Press; 1962.

Imen K, Marrot B, Amar RB. Decolourization of the reconstituted dye bath effluent by commercial laccase treatment: Optimization through response surface methodology. Chem Eng J 2010;156:121-33.

ISTA. International rules for seed testing. Seed Sci Technol 1985;13:299-355.

Jones DL, Kielland K. Amino acid, peptide and protein mineralization dynamics in a taiga forest soil. Soil Biol Biochem 2012;55:60-9.

Karimi N. Comparative phytoremediation of chromium-contaminated soils by Alfalfa (*Medicago sativa*) and *Sorghum bicolor* (L) Moench. Int J Sci Res Environ Sci (IJSRES) 2013;1:44-9.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

Mace ME. Histochemical localization of phenols in healthy and diseased tomato roots. Phytochem 1963;16:915-25.

Pikovskaya RE. Mobilization of phosphorous in soil in connection with vital activity of some microbial species. Microbiologiya 1948;17:362-70.

Ramakrishnan S, Rajan R. Text Book of Medical Biochemistry. 2nd ed. New Delhi, India: Orient Longman; 1994. p. 582.

Waldi D. In: Stahl E, editor. Thin-Layer Chromatography: A Laboratory Handbook. New York. Academic Press, Inc.; 1951. p. 491.

Wang C, Yediler A, Linert D, Wang Z, Kettrup A. Toxicity evaluation of reactive dye stuff, auxiliaries and selected effluents in textile finishing industry to luminescent bacteria vibrio fisheri. Chemosphere 2002;46:339-44.

Wiwart M, Mos M, Wojtowicz W. Studies on the imbibition of triticale kernels with a different degree of sprouting, using digital shape analysis. Plant Soil Environ 2006;52:328-34.

Yoshida S, Tracey M, Freeman DC. Developmental instability as a biomonitor of environmental stress. In: Butterworth FM, editor. Biomonitors and Biomarkers as Indicators of Environmental Change. New York, NY: Plenum Press. 1993 p. 313-37.

Xie H, Li C, Xu J, Li H. The mechanism of Evans blue removal by sunflower. J Chem Pharm Res 2014;6:327-31.