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RESEARCH ARTICLE

BIOMETRIC ANALYSIS AND PHYTOHORMONE PRODUCTION IN SESBANIA GRANDIFLORA'S RHIZOSPHERE SOILWITH BIOTREATED (*PSEUDOMONAS FLUORESCENS AND AZOSPIRILLUM* SP.) SILK DYEING EFFLUENT

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ABSTRACT

The research work has been focused on the analysis ofPGPR in raw silk dyeing effluent and biotreated effluent by *Pseudomonas fluorescens* and *Azospirillum sp.* The Green leafy vegetable *Sesbania grandiflora*sowed seedswas treated withraw silk dyeing effluent, *Pseudomonas fluorescens* and *Azospirillum sp.* separately. After 45th days the biometric parameters such as First cotyledon, Seed germination (%), Seedling length (cm), Number of Leaves plant^{-1} , Surface area of leaves (sq/cm), Root length (cm), Shoot length (cm), Root shoot ratio, Root mass (g), Dry matter (%),Number of Seed bunch plant^{-1}, Distance between the nodes (cm) were analysed. The phytohormone such as Indole acetic acidand Gibberellic acid were also analysed. The IAA production by *Pseudomonas fluorescens* and *Azospirillum sp.,* in the silk dyeing industrial effluent was found to be 78µg 25ml^{-1} and 70.4µg 25ml^{-1} respectively. The biofertilizers *Pseudomonas fluorescens* and *Azospirillum sp.* synthesizes about 6.9µg 25ml^{-1} and 7.1µg 25ml^{-1} of GA_3in the Whereas the untreated silk dying effluent was hardly seen the phytohormone presence. The study confirms that the selected biofertilizers (*Pseudomonas fluorescens* and *Azospirillum sp*.) produced more IAA than GA_3. Thus *Pseudomonas fluorescens* biotreated silk dyeing industrial effluent can be used for the growth of plants.

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INTRODUCTION

Soil is a dynamic living matrix and it plays a major role. Phytohormones are the chemicals that regulate cellular activities. The rhizobacteria help in regulating it. They are essential for the growth and development (Lia Jia Qu *et al*., 2011). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces which associates to improve the quality of plant.(Villacieros *et al.,* 2003). The ePGPR are the external plant growth promoting rhizobacteria present in the rhizosphere (Viveros e*t al.,* 2010).These microbes are having the capacity of biodegradation of complex chemical compounds and also they act as PGPR (Plant growth promoting rhizobacteria) which produce antibiotics as well as secondary metabolites such as siderophore, phytohormones, volatile compounds and hydrogen cyanide (HCN).

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The *Azospirillum sp.*, and *Pseudomonas sp.,* belong to the plant growth promoting rhizobacteria that are the bacteria capable of promoting plant growth by colonizing the plant's root (Prakash and Karthikeyan, 2013). The growth of plants and its healthy parts indicate that the plant rhizosphere soil has PGPR and it has produced phytohormones that are believed to be related to their ability to stimulate plant growth. So the biometric parameters can also be documented. Indole-3-acetic acid (IAA) is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement. This hormone is very commonly produced by PGPR. Gibberellins (GA_3) are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury, 1994). Furthermore, the plant roots seem to be an ideal environment for the degradation of organic compound as a result of numerous mechanisms. The plant root system allows the rapid movement of water and gases through soil structure. It also provides a biologically active soil region (i.e) Rhizosphere enhances the microbial activity and the contaminant

bioavailability which is the promising green technology for remediation of contaminated soil (Joner *et al*., 2007).

MATERIALS AND METHODS

Collection of Seeds: Seeds of agate (*Sesbania grandiflora*) were collected from Superseeds Nursery, Coimbatore.

Seed sowing and maintenance of plants: About 20 seeds were sown in each pot and were allowed to germinate. Neem cake was mixed with water and poured around the pots as pest control. Fresh water, silk dyeing effluent of different concentrations (25%, 50%, 75% and 100%) and crude silk dyeing effluent treated with *Pseudomonas fluorescens* have been used in Phase 1, Phase 2 and Phase 3 respectively. After germination, 100% moisture condition was maintained throughout the study.

Harvest methodology: The plants were harvested on the 45th day without any damage. The adhering soil particles were removed by washing gently with water and the water droplets were removed by blotting with the filter paper. Then these plants were subjected to various analysis like biometric parameters and phytohormone quantification.

Biometric observations: Biometric observations of the GLV plants were recorded on the 45th day of growth. Measuring plants' vegetative growth is by capturing enough data on the overall health of the plants.

First cotyledon: The number of days from the date of the seed sowing till the first cotyledon emerged was recorded.

Germination percentage: The seeds that germinated from 20 sown seeds were counted and the percentage germination was calculated.

Seedling length: The height of the uprooted plant from the tip of the root to top of main plant stem at 45th day of growth was measured and was expressed as cm per plant.

Number of leaves: Every visible leaf on the plant was counted, including the tips of the new leaf just beginning to emerge. The plant was kept over some graph paper to avoid counting errors. The number of leaves of each plant was counted on the 45th day after sowing and was expressed as number of leaves per plant.

Surface area of leaves: The leaves of different plants were hand-drawn onto the grid paper. The 2 cm grid paper showed both the basic leaf parts (blade and petiole). Since the petiole did not contribute much to photosynthesis, it was removed from the other drawings. The squares covered by each leaf were counted and the surface area was estimated. For each calculated surface area, whole squares located within the leaf area drawing were identified and multiplied by the appropriate area of each grid size (i.e. the area for each square using 2 cm grid paper = 4 cm^2). Squares which included part of the leaf surface were added up and then divided by 2 since only part of the surface was included within the square (Gerber and Mccool, 2014).

Root length: The Root length was measured from the crown region of the plant to the tip of the root and expressed as cm per plant.

Shoot length: The Shoot length was measured from the point of first cotyledonary node to the tip of the longest leaves and expressed as shoot length in cm per plant.

Root - Shoot ratio: The harvested plants were dried in an oven overnight at low heat. The plants were left to cool in a dry environment by keeping it in a ziploc bag (which will keep the moisture out) whereas in a humid environment the tissue will take up water). Once the plants got cooled, they were weighed. The root and the shoot were separated and weighed. The Root - Shoot ratio was calculated

Root - Shoot ratio = Dry weight of roots / Dry weight of shoot

Root mass: The plant from the soil was removed. The adhering soil particals were removed by washing gently with water and the water droplets were removed by blotting with the filter paper and the roots were separated, weighed and expressed as grams per plant.

Dry matter production: The plants were dried in an oven at low heat overnight. The plants were cooled in a dry environment, than in a humid environment. Once the plants got cooled, weighed and calculated by the formula.

Total Dry Matter (Total DM) = (Dry weight of the plant / Initial weight of the plant) x 100.

Number of Seed bunch: The number of the seed bunch was counted and recorded as seeds per plant.

Distance between nodes: The distance between the nodes of the plant were measured and expressed in cms.

Quantification of Phytohormones (IAA, GA_3): A loop of culture from the cultivated medium of plant growth promoting *Azospirillum sp.* and *Pseudomonas fluorescens* was inoculated separately to each flask of 100% crude silk dyeing industrial effluent with freshly prepared, sterilized solution of L-tryptophan. It was incubated at 37^{o}C in dark for seven days. After incubation, the cultures were centrifuged at 6,000 rpm for 5 minutes to remove the bacterial cells. The supernatant was brought to pH 2.8 with 1N HCl (Megala and Elango, 2013). The *invitro* production of phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA_3) was determined by the procedures as follows. The indole acetic acid (IAA) content was determined by the method of Gordon and Paleg, (1957).The giberrellic acid content was determined by the method of (Borrow *et al.,* 1955).

RESULTS AND DISCUSSION

Biometric analysis of *Sesbania grandiflora* grown in the silk dyeing effluent: Table 1 and Plate 1 respectively represent the biometric parameters and growth of the *Sesbania grandiflora* grown in different treatments of the silk dyeing effluent. Without any disparity all the plants had their first germination within 3 days similar to plants grown in fresh water. It was clear from the table 1 that the percentage seed germination, the seedling length, number of leaves per plant, the root length, the shoot length and the dry matter content of *S.grandiflora* plants were gradually reduced with different treatments of silk dyeing effluent. The parameters such as surface area of the leaves, the root shoot ratio, the root mass and the distance between the nodes of *S.grandiflora* plants did not vary much between the different treatments of the effluent.

Table 1. Biometric parameters of *S .grandiflora*(SGA) grown in different treatments of the silk dyeing effluent (SDE)

Parameters	SGA with Fresh water	SGA with *Pseudomonas fluorescens* treated SDE	SGA with *Azospirillium sp.,* treated SDE	SGD 100% with SDE
First cotyledon		Within 3 Days		
Seed germination (%)	50.0±14.7	50.0±13.8	40.0±10.8	10.0±2.7
Seedling length (cm)	43.0±0.8	22.0±0.6	17.5±0.1	12.0±2.0
Number of Leaves plant^{-1}	140.0±0.8	120.0±8.1	111.0±4.3	20.0±0.3
Surface area of leaves (sq/cm)	9.0±0.02	9.0±0.04	8.0±0.07	5.0±0.05
Root length (cm)	11.0±0.3	10.0±1.1	8.6±0.3	4.0±0.7
Shoot length (cm)	32.0±4.1	12.0±1.6	8.9±0.4	5.0±1.1
Root shoot ratio	1.1	1.0	0.9	0.7
Root mass (g)	4.0±0.5	3.5±0.2	3.1±0.4	1.0±0.1
Dry matter (%)	77.7±0.8	64.4±0.28	61.2±1.03	35.7±0.7
Number of Seed bunch plant^{-1}		-		
Distance between the nodes (cm)	4.0±0.80	4.2±0.4	3.9±0.5	3.1±0.7

Values are the mean of three replicates ± SD; Note- S.grandiflora did not produce seeds and flowers within the experimental period; SGA: Sesbania grandiflora grown in different treatments; SDE: Silk dyeing effluent

Table 2. Production of phytohormones by selected biofertilizers

Inoculum in Silk dyeing industrial effluent	IAA(µg 25ml^{-1})		GA_3 ((µg 25ml^{-1})	
	Untreated raw Silk dyeing	Biotreated Silk dyeing industrial effluent	Untreated raw Silk dyeing	Biotreated Silk dyeing industrial effluent
Pseudomonas fluorescens	0	78.0	0	6.9
Azospirillum sp.,	0	70.4	0	7.1

Plate 1. *Sesbania grandiflora* grown in different treatments of the silk dyeing effluent

***Sesbania grandiflora* in fresh water**

SGA with *Pseudomonas fluorescens* treated SDE

SGA with *Azospirillium sp.,* treated SDE

***Sesbania grandiflora* in 100% effluent**

Plate 2. Production of IAA and GA_3 by *P. fluorescens* and *Azospirillum sp*., in Silk dyeing effluent

Thus in the growth studies of selected GLV, the biometric parameters were affected with raw silk dyeing effluent whereas the treatments with *Pseudomonas fluorescens* showed the positive changes in the biometric parameters compared with *Azospirillium sp.,* treated Silk Dyeing effluent. Growth analysis is functions of a large number of metabolic processes, which are affected by the environmental factors (Caglar *et al.,* 2010). The effluent water and polluted soil decreases the growth of vegetables, this may be due to their toxicity (Sangannavar and Kalshetty, 2011). Thus the *Sesbania grandiflora* (SGA) with *Pseudomonas fluorescens* treated Silk Dyeing effluent (SDE) can be used comparatively instead of fresh water.

Production of phytohormones by selected biofertilizers (*Pseudomonas fluorescens* and *Azospirillum sp.*): Table 2 and Plate 2 illustrate the level of phytohormones (Indole acetic acid and Gibberellic acids) in silk dyeing industrial effluent inoculated by *Pseudomonas fluorescens* and *Azospirillum sp*.
The IAA production by *Pseudomonas fluorescens* and *Azospirillum sp.,* in the silk dyeing industrial effluent was found to be 78µg $25ml^{-1}$ and 70.4µg $25ml^{-1}$ respectively. The biofertilizers *Pseudomonas fluorescens* and *Azospirillum sp.* synthesizes about 6.9µg $25ml^{-1}$ and 7.1µg $25ml^{-1}$ of GA_3.Whereas the untreated silk dying effluent was hardly seen the phytohormone presence. Thus the study confirms that the selected biofertilizers (*Pseudomonas fluorescens* and *Azospirillum sp*.) produced more IAA than GA_3.The beneficial bacteria referred as plant growth promoting bacteria (PGPB), which support the growth via production of phytohormones (Bai *et al.,* 2002). The most efficient PGPB studied for their ability to produce phytohormones are *P. putida*, *P. fluorescens*, *Azosprillum* spp. and *Bacillus* spp. (Ahamed, 2007). Hence the present study compares the phytohormones IAA and GA_3 of the selected biofertilizers *Pseudomonas fluorescens* and *Azospirillum sp.* and *Pseudomonas fluorescens* is recommended as the better phytohormones synthesis which in when used as remediating the silk dying effluent (Sumayya *et al.,* 2014 a & b)grown in normal, effluent exposed and effluent biotreated conditions.

REFERENCES

Ahamed, I.H. 2007. Efficacy of rhizobacteria for growth promotion and biocontrol of *Pythium ultimum* and *Fusarium oxysporum* on sorghum in Ethiopia and South Africa, Ph.D Thesis, Department of Microbiology and Plant Pathology, University of Pretoria, 6.

Bai, Y., Aoust, F. D., Smith, D. L. and Driscoll, B. T. 2002. Isolation of plant growth promoting *Bacillus* strains from soybean root nodules, *Canadian Journal of Microbiology,* 48: 230-238.

Borrow, A.P.W., Brain, U.E., Chester, P.J., Curtis, H.G., Hemming, E.C., Jeffereys, R.B., Lloyd, I.S.,Nixon, G.L.F., Norris, L. and Radley, N. 1955. Gibberellic acids a metabolic product of the fungus *Gibberella fujikuroi* some observations on its production and isolation, *Journal of Food Science and Agriculture,* 6: 340-348.

Caglar, O., Hasan, T.S., Ihsanullah, D. and Oguz, F. 2010. Relationship between some plant growth parameters and grain yield of chickpea, *Cicer arietinum* L.) during different growth stages, 15:79-83.

FB Salisbury. The role of plant hormones. In: Wilkinson RE, eds) Plant–Environment Interactions. Marcel Dekker, New York, USA, 1994, 39-81.

Gerber, D. and McCool, J. 2014. Finding the Surface Area of a Leaf, http://sciencenetlinks.com/student-teacher-sheets/surface-area-leaf.

Gordon, S.A. and Paleg, L.G. 1957. Observations on the quantitative determination of indole acetic acid, *Physiological Plantarum,* 10: 39-47.

Joner, E.J., Johannes, A., Loibne, A.P., De La Cruz M.A., Szolar O.H., Portal J.M. and Le yual, C.,, 2007. Rhizosphere effects on Microbial community structure and dissipation and Toxicity of Polycyclic aromatic hydrocarbon, PAHs) in spiked soil, *Environmental Science and Technology,* 35: 2773.

Li Jia Qu, Yunde Zhao, 2011. Plant hormons: Metabolism, signaling and crosstalk, *Journal of interactive plant biology,* 53(6),pp 1744-54.

Megala, S. and Elango, R. 2013. Phytohormones production by plant growth promoting rhizobacterial isolates in *Gloriosa superba* .L., *Indian Journal of Applied research,* 3: 1-3.

Prakash, P. and Karthikeyan, B. 2013),Isolation and purification of Plant Growth Promoting Rhizobacteria, PGPR) from the rhizosphere of *Acorus Calamus* grown soil, *Indian Streams Research Journal,* 3:1-13.

Sangannavar, M. C. and Kalshetty, B. M. 2011. Morphological parameters and chlorophyll content of green vegetable plants available around Jamkhandi, *Current World Environment,* 6: 67-70.

Sumayya A.R, Sivagami Srinivasan, 2014a. Study of Physical Parameters of Untreated and Treated Silk Dyeing Effluent by *Azospirillum sp*., and *Pseudomonas fluorescens*, Proceedings of "Global opportunities for recent developments in science and technology", pp D-277 – D-279, ISBN 9789382323266.

Sumayya A.R, Sivagami Srinivasan, 2014b. Analysis of Chemical characteristics in untreated and biotreated Silk dyeing effluent by *Azospirillum sp*. and *Pseudomonas fluorescens*, *International Journal Applied Biological and Chemical sciences,* 3(1): 176-179, ISSN: 2277 - 4688.

Villacieros, M., Power, B., Sanchez-Contreras, M., Loret, J., Oruzebal, R.I., Martin, M., Franandez Pinas, F., Bouile, I., Whelan, C., Dowling, D. N. and Rivilla, R. 2003. Colonization behaviour of *Pseudomonas fluorescens* and *Sinorhizobium meloti* in the alfalfa, *Medicago sativa*) rhizosphere, *Plant and Soil,* 251: 47-54.

Viveros OM, Jourqura MA, Crowley DE, Gajardo G, Mora ML, 2010. Mechanism and practical consideration involved in the plant growth promotion byrhizobacteria , *J Soil Sci Plant Nutr*., 10:293-319.

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