

RESEARCH ARTICLE

HISTOLOGICAL STUDIES OF THE LEAF TISSUES IN SELECTED GLV'S OF FRESH WATER, CRUDE SILK DYEING EFFLUENT AND BIOTREATED EFFLUENT

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ABSTRACT

The research work has been focused on the morphological studies of the leaf tissues in the green leafy vegetables (GLVs) namely mustard (*Brassica juncea*), fenugreek (*Trigonella foenum*), sirukeerai (*Amaranthus polygonoides*), araikeerai (*Amaranthus tristis*) and agati (*Sesbania grandiflora*). The prepared sections from the GLVs grown on fresh water as control revealed properly formed tissues. There was no pathological death observed for all the tissues. A clear view was not revealed in leaves of the GLVs exposed to the crude silk dyeing effluent. In leaf tissues of GLVs grown in biotreated effluent were histologically observed and they are similar as that of the GLVs grown in fresh water. The study confirms the effluent's impact has been countered by the biofertilizers of *Pseudomonas fluorescens* as it has the ability to decolorize the silk dyeing effluent. Thus *Pseudomonas fluorescens* biotreated silk dyeing industrial effluent can be used for the growth of plants.

Key words: *Pseudomonas fluorescens*, *Azospirillum* sp., Silk dyeing effluent, mustard, *Brassica juncea*, fenugreek, *Trigonella foenum*, sirukeerai, *Amaranthus polygonoides*, Araikeerai, *Amaranthus tristis*, agati and *Sesbania grandiflora*.

INTRODUCTION

The textile industry accounts for two-third of the total dyestuff market. More than 10,000 different textile dyes with a predictable annual production of 7×10^5 metric tons are commercially available worldwide (Aksu, 2005). The color in watercourses is accepted as an aesthetic problem rather than an eco-toxic hazard. Therefore, the public seems to accept blue, green or brown color of rivers but the 'non-natural' colors such as red and purple usually cause most concern. The polluting effects of dyes against the aquatic environment can also be the outcome of the toxic effects due to their long time presence in the environment. (Mathurand Bhatnagar, 2007). 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 environmental authorities have begun to target the textile industry to clean up the wastewater that is discharged. The environmental issues related with the the residual dye content or the residual color in treated textile effluents are always a concern for each textile operator that directly discharges the effluents into the atmosphere (Zaharia *et al.*, 2011). The effluent discharge from the silk dyeing industries is common in Salem district. The major issue that has caused failure to small units is the high cost of running a plant, lack of electricity, non-payment of user charges of reagent for the treatment of effluent.

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Also the current effluent treatment plants fail when the operation does not involve chemicals at the right time and in the right quantity (Sumayya *et al.*, 2015). The objective of this paper is to provide ecofriendly environment of dyeing unit effluents that positively affect the lives of the poor artisans as well as their communities who are unable to establish treatment plants in Salem district. Many fluorescent *Pseudomonas* strains have been reported as plant growth enhancing beneficial rhizobacteria. They are studied widely in agriculture for their role in crop improvement as they stimulate plant growth either by producing plant growth promoting hormones, fixing atmospheric N_2 or suppressing plant pathogens. The ubiquitous rhizosphere microflora forming symbiosis with plant roots, act as biofertilizers, bioprotectants and biodegraders (Khan Abdul, 2011). Based on the highest percentage decolorization the *Pseudomonas fluorescens* (Sumayya *et al.*, 2015) was selected for the further studies. Furthermore, the leaf morphology of various Green leafy vegetables (GLVs) are subjected to various treatments such as fresh water, crude Silk dyeing effluent and Biotreated effluent (by *Pseudomonas fluorescens*).

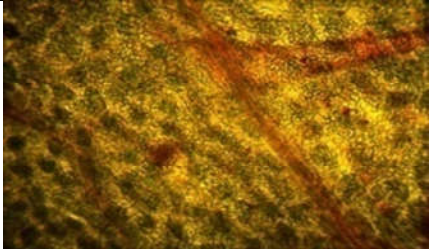
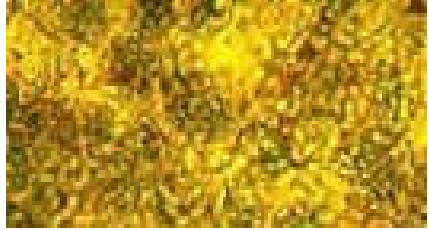
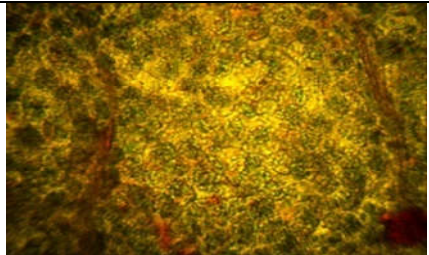

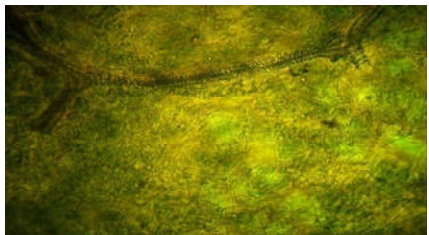
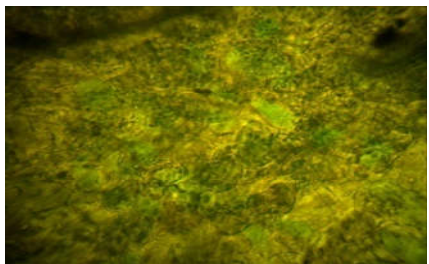
MATERIALS AND METHODS

Five green leafy vegetables namely mustard (*Brassica juncea*), fenugreek (*Trigonella foenum*), sirukeerai (*Amaranthus polygonoides*), araikeerai (*Amaranthus tristis*) and agati (*Sesbania grandiflora*) were collected from Superseeds Nursery, Coimbatore.

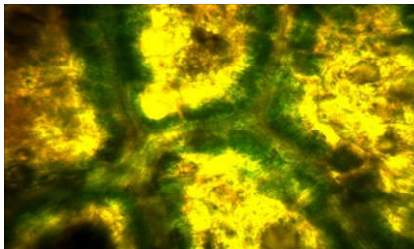
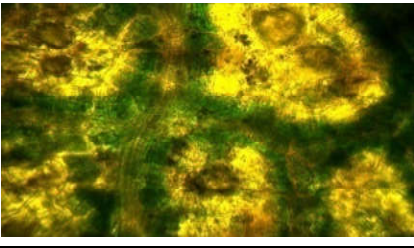
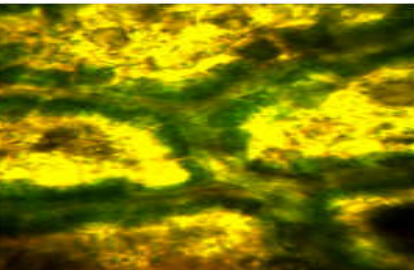
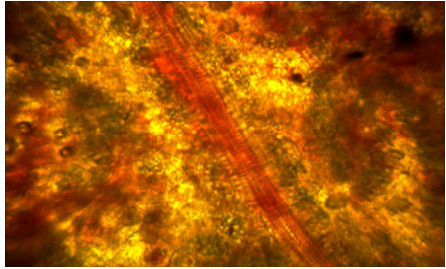
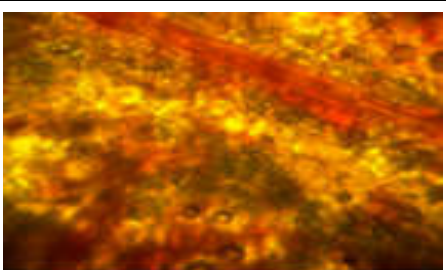
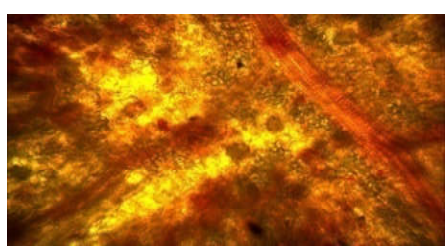
Seed sowing and maintenance of plants: About 20 seeds were sown in each pot and were allowed to germinate. Neem cake was mixed with water and poured around the pots as pest control.

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.

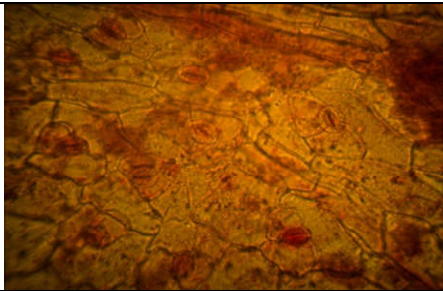
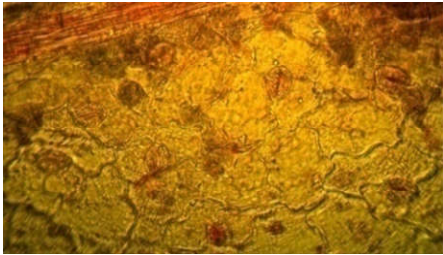
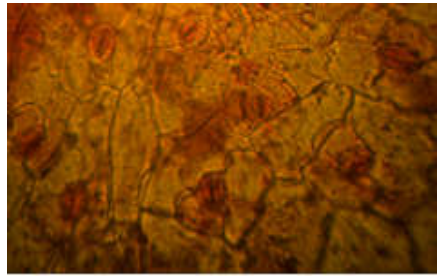
Plate 1. Histological studies of the selected GLVs

<i>B. juncea</i>	Histological view	Observation
Plate 1.1		
Leaf of plants from fresh water		Clear cells with the chloroplast were seen in the leaf of <i>B. juncea</i>
Leaf of plants from crude silk dyeing effluent		A blurred appearance was viewed in the cells of the leaf
Leaf of plants from Biotreated effluent		The clear cells visualized in the leaf of <i>B. juncea</i> with plasmadesmata
<i>T. foenum</i>	Histological view	Observation
Plate 1.2		
Leaf of plants from fresh water		The plasmadesmata which acts as a channel was observed
Leaf of plants from crude silk dyeing effluent		The chlorophyll was viewed but the plasmadesmata which acts as a channel was disconnected
Leaf of plants from Biotreated effluent		The plenty of chloroplasts were observed which in turn produces the green color

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<i>A.polygonoides</i>	Histological view	Observation
Plate 1.3		
Leaf of plants from fresh water		The chlorophyll pigments cells were seen at the edge of the cell
Leaf of plants from crude silk dyeing effluent		The leaf has chlorophyll in the edges with some black spots of dye interactions inside the cell
Leaf of plants from Biotreated effluent		The chloroplast cells were seen at the corner
<i>A.tristis</i>	Histological view	Observation
Plate 1.4		
Leaf of plants from fresh water		The veins were seen clearly with chloroplast
Leaf of plants from crude silk dyeing effluent		An unclear view was observed.
Leaf of plants from Biotreated effluent		The chloroplast and plasmodesmata were viewed clearly
<i>S.grandiflora</i>	Histological view	Observation

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<i>S.grandiflora</i>	Histological view	Observation
Plate 1.5		
Leaf of plants from fresh water		The chloroplasts were observed within the cell
Leaf of plants from crude silk dyeing effluent		Few chloroplasts were observed
Leaf of plants from Biotreated effluent		Chloroplasts were viewed within the intact cell

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.

Histological studies: The leaf tissues of *Brassica juncea*, *Trigonella foenum*, *Amaranthus polygonoides*, *Amaranthus tristis* and *Sesbania grandiflora* in all the three phases on the 45th day were stained with Giemsa and Saffranin as described in Saffranin O staining (Jong Kwiton, 1970) and Giemsa staining (1904). The images were examined under the microscope and snapped using the Motic digital camera associated with Motic Image plus 2.0 software.

RESULTS AND DISCUSSIONS

Histological studies of the leaf tissues of GLVs grown indifferent treatments: Plate 1 depicts the histology of the leaf tissues of the selected plants of GLVs grown in fresh water under normal conditions, silk dyeing effluent and biotreated effluent. In leaf tissues of GLVs grown in fresh water, the sections of the leaf tissues showed a fine structural integrity which correlates with the findings of Uaboi-Egbenni *et al.* (2009) which stated that the prepared sections from the plant grown on control revealed properly formed tissues.

There was no pathological death observed for all the tissues. In leaf tissues of crude effluent, the microscopic view in all the GLVs were histologically observed and recorded. A clear view was not revealed in leaves of the GLVs exposed by the effluent. These cytological abnormalities may be due to the presence of higher concentrations of the chemicals in the effluent that may disturb the normal cell cycle (Shobha, 2004). Clumpiness was revealed in the histological view of leaves of the GLVs affected by the effluent. In leaf tissues of GLVs grown in biotreated effluent, the sections of the leaf GLVs were histologically observed and they are similar as that of the GLVs grown in fresh water. It proves that the effluent's impact has been countered by the biofertilizers of *Pseudomonas fluorescens* as it has the ability to decolorize the silk dyeing effluent. Many microorganisms belonging to different taxonomic groups such as bacteria had been reported for their ability to decolorize the dyes (Chen *et al.*, 2003). Hence the present study explains that the histological studies of leaf tissues of five green leafy vegetables grown in all the treatments and the biotreated effluent treated GLVs is viewed as similar as the GLVs grown in the fresh water. Thus the morphological characteristics in the leaf of the GLVs grown in biotreated effluent consider to be as same as the GLVs grown in fresh water which in when used as the *Pseudomonas fluorescens* was found to be more effective in degrading silk dyeing effluent (Sumayya *et al.*, 2014).

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