ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by Pharmascope Publications Journal Home Page: www.pharmascope.org/ijrps

Fourier Transform Infrared (FTIR) Spectroscopy and High-Performance Liquid Chromatography Analysis of *Brassica juncea* (Mustard) and Silk dyeing effluent's impact on the spectral studies

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Article History:

Received on: 13.10.2018
Revised on: 16.03.2019
Accepted on: 18.03.2019

Keywords:

Pseudomonas fluorescens,
Azospirillum sp.,
Silk dyeing effluent,
Amide,
Isocyanides,
UV,
FTIR,
HPLC analysis

ABSTRACT

The exclusive, low-cost nominal technology for the meagre entrepreneurs of Silk dyeing effluent has been planned and executed. Environmental pollutants exit like Silk dyeing effluent are destructive and needs a high-cost Common Effluent Treatment Plant (CETP) to achieve Zero effluent discharge limits which are not reasonably priced for a low venture capitalist. The Green leafy vegetable *Brassica juncea* sowed seeds were treated in pot study with fresh water, raw Silk dyeing effluent and Biotreated effluent (with *Pseudomonas fluorescens* and *Azospirillum sp.* biofertilizers separately). After 45th days the GLV's extracts *Brassica juncea* were grown in fresh water (BJN), in crude effluent (BJE) and in biotreated effluent (BJT) were subjected to UV, FTIR and HPLC analysis. Thus, from the functional group studies by FT-IR, the alcohol, alkane, alkyl halide and amine groups were found in GLV irrespective of the treatments, even in crude effluent, the plants managed to synthesize these organic compounds. The isocyanide group was found only in *B. juncea*, grown in fresh water, which was unable to synthesize isocyanide group in plants grown in crude effluent and biotreated effluent. While the biotreated *B. juncea* methanolic extracts had shown two peaks of similar to the freshwater *B.juncea* methanolic extracts whereas the crude effluent had its effect in HPLC Analysis. So it clearly indicates that the effluent's effects have been encountered by the *Pseudomonas fluorescens*.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i2.419

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INTRODUCTION

Effluent discharged into the environment may have a severe negative impact on the quality and the life forms of the receiving water body when discharged untreated or partially treated. (Okoh, 2007). The textile business interprets on behalf of two-thirds of the total dyestuff market. More than 10,000 diversified textile colorings with a probable annual making of $7x10^5$ metric tons are commercially reachable worldwide (Aksu, 2005).

Wastewater outlets in printing and dyeing units are frequently rich in color, containing residues of dyes and chemicals and needs proper treatment

before being released into the environment. Without adequate treatment, these dyes can remain in the environment for a longer period (Allegre *et al*., 2006). Due to their synthetic nature and structure mainly aromatic, most of the dyes are non-biodegradable, having carcinogenic action or causing allergies, dermatitis, skin irritation or different changes in tissues (Bornick and Schmidt, 2006). In addition to being toxic, the dye effluents also include chemicals that are carcinogenic, mutagenic or teratogenic to various organisms (Mathur and Bhatnagar, 2007).

The silk dyeing manufacturing unit comprises water rigorous processes that need a large volume of good quality of water. Some of the small scale dyeing units are demolishing storage facilities for hazardous waste, but apt disposal facilities are not available, which pollute water sources as they are also released into the drainage system and cause a crucial hazard to the health of native communities, in addition, poisoning the drinking water (Nupur, 2009). The total quantity of wastewater produced from dyeing activity is recounted to be about 600 m^3/day and from the bleaching activity is 100 m^3/day (Mohana *et al.,* 2008).

At contemporaneous, the Common Effluent Treatment Plant (CETP) established in major places involves some physicochemical methods such as filtration, specific coagulation, absorption by means of activated carbon, chemical flocculation, precipitation, ozone ionizing radiation, ultra-filtration etc. The wastewater disposal going on agricultural land is the potentially phytotoxic nature of organic wastes, mainly as a consequence of a combination of factors such as high salinity or excess of ammonium ions and organic compounds (Lazarova and Bahri, 2005). Wastewater from the textile industries constitutes a threat to the environment in large parts of the world (Nilsson *et al.,* 2006).

The ever-present nature of bacteria makes invaluable tools in the effluent biotreatment. Bacteria are essential to the breakdown of the toxic component of the effluent. Microbial decolorization and degradation is an environmentally friendly and cost reasonable alternative to chemical decomposition process (Watson and Cichra, 2006).

Biological systems adeptly lessen silk dyeing wastewaters because they are cheaper and produce lesser amounts of slush, as sludge treatment and disposal is a vibrant component in the global cost of treatment. However, the advanced biological processes have established increasing attention due to low cost, effectivity, less sludge generation and eco-friendly nature (Lotito *et al.,* 2012).

The Green leafy vegetable *Brassica juncea* is commonly known as "kadugu", selected as an experimental plant for the analysis.

The Indian Mustard *Brassica juncea* is from the family Brassicaceae commonly used as the oil source, a green vegetable, with medicinal properties (Manohar *et al.,* 2009). The leaves, the seeds and the stem of this mustard variety are edible. Cultivars of *Brassica juncea* are grown as greeneries and for the production of oilseed (Heimler, 2007). It is being grown for its seeds which yield an essential oil and condiment. Mustard oil is one of the major edible oil in India (Jeffery *et al.,* 2008). Brassica is highly regarded for its nutritional value. They provide high amounts of vitamin C and soluble fibre and contain multiple nutrients with potent anticancer properties (Erickson, 2008). Indian mustard is a folk remedy for arthritis, foot ache, lumbago and rheumatism (Choi and Lee, 2009). The seed is used for tumours in China. Leaves are applied to the forehead to relieve headache (Fitzjohn, 2007). In Korea, the seeds are used for abscesses, colds, lumbago, rheumatism and stomach disorders. Chinese eat the leaves in soups for bladder inflammation and haemorrhage. Mustard oil is used for skin eruptions and ulcers (Maity *et al.,* 2008).

Indian mustard is a source of various micronutrients as well as antioxidants, carotenes and it acts as a source of nutraceuticals (Kumar *et al.,* 2011), help to lower diabetic and comorbid anxiety disorder (Thakur *et al.,* 2013) and various volatile compounds (Kessler *et al.,* 2002), produces medicines such as stimulants, diuretics, and expectorants (Farrell, et al., 1985).

MATERIALS AND METHODS

Assemblage of Seeds

The Mustards seeds (*Brassica juncea*) were obtained from Superseeds Nursery, Coimbatore.

Soil groundwork for the study

In Phase 1 and Phase 3, *Brassica juncea* GLV were grown with four replicates. In phase 2, three pots for each of the four different concentrations (25%, 50%, 75% and 100%) were used. The biofertilizer, *Pseudomonas fluorescens* was mixed at the rate of 5 tonnes ha^{-1} with crude effluent and used in Phase 3. The bacterial concentration of the biofertilizer was 10^8 Colony forming units (CFU) ml^{-1}. The red soil and the sand were mixed at the ratio of 3:1. Each pot was filled with 7 kg of soil.

Seeding and upkeep 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.

Freshwater, 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 GLV was reaped on the 45th day without any injury. The standby soil particles were aloof by washing gently with water, and the water droplets were removed by blotting with the filter paper. Then these GLVs were separately dried with a division of different phases and subjected to various analysis as follows.

Identification of functional groups and compounds in selected GLV of different treatments using spectroscopic and chromatographic techniques

- UV visible analysis was carried out on the methanolic extract of the selected GLV Extract grown in Fresh Water, effluent and Biotreated water.
- FT-IR analysis of the selected GLV grown in fresh water, crude effluent and biotreated effluent and selected dyes in silk dyeing effluent was performed.
- HPLC analysis was carried out in the selected GLV grown in fresh water, crude effluent and biotreated effluent and untreated silk dyeing effluent and biotreated effluent.

The detailed experimental design of the four phases of the study are as follows:

UV visible analysis of selected GLVs

The *Brassica juncea* plants were air dried, and 20gms of finely powdered material of each were taken in a thimble and extracted using 200ml of HPLC grade methanol in Soxhlet apparatus. The methanolic extracts of selected GLVs were subjected to Bio-nano UV visible spectrophotometer at a different wavelength to find its maximum absorbance peak.

FT-IR analysis of selected GLVs plants and selected dyes

FT-IR (Fourier Transform Infrared) analysis provides spectral information that is essentially a molecular fingerprint for organic, polymeric and in some case inorganic materials. This technique is extremely useful for identifying base polymer compositions and organic contaminants. The FT-IR spectrum of the unknown material can be compared for "best matches" with libraries of spectra that have been catalogued for known materials. The FT-IR analysis was carried out for the selected plants of the GLVs grown in fresh water, crude effluent and biotreated effluent to identify the organic compounds.

1) In Phase 1, the selected GLV Mustard (*Brassica juncea*) treated with the fresh water were removed on the 45th day, air dried, powdered and subjected to FT-IR.
2) In Phase 2**,** The selected plants were grown in crude silk dyeing effluent until 45 days. The effluent exposed plant was air dried, powdered and subjected to FT-IR.
3) In Phase 3, The selected GLV was treated with the biotreated silk dyeing effluent. The effluent bio treated plants were air dried, powdered which was subjected to FT-IR.

HPLC analysis of selected GLVs.

Sample preparation for HPLC analysis

1) In Phase 1, the selected *Brassica juncea* treated with the fresh water, on the 45th day were removed, air-dried and ground into powder. The HPLC grade methanol was purchased from Fischer Scientific & Co. 20 grams of each dried powder sample was weighed and then packed in Whatmann filter paper placed in a thimble of Soxhlet apparatus. The HPLC grade methanol of 200 ml each was taken in a round bottom flask, and the extract was prepared, then stored in the dark at 4°C which was subjected to HPLC analysis at 450 nm.
2) In Phase 2, the selected plant was treated with the crude silk dyeing effluent. At the end of the 45th day, the effluent exposed plants were removed, air-dried and powdered. The methanolic extracts of the plants were obtained from the Soxhlet apparatus as in phase 1 and subjected to HPLC analysis at 450 nm.
    a) The collected crude silk industrial effluent and degraded effluent by *Pseudomonas fluorescens* was filtered and subjected to HPLC analysis at 510 nm.
3) In Phase 3, the selected plants were treated with the biotreated silk dyeing effluent. The plants grown in biotreated effluent were uprooted on the 45th day and air dried and powdered. The methanolic extracts of selected plants were obtained from the soxhlet apparatus as in phase 1 and subjected to HPLC analysis at 450 nm.

All the peaks were analyzed between the phases and were compared with the standards of pigments (chlorophyll C2, chlorophyll C3, chlorophyll B, carotene), an alkaloid (caffeine) and monosaccharides (glucose, fructose, mannose and galactose) which was subjected to HPLC analysis at 450 nm.

Chromatographic conditions

The chromatographic system was equipped with column C18 with a 3µl particle size (50×4.6 mm I.D) and detector UV- VIS model SPD 20A at specific nanometer at a flow rate of 1ml/min. The solvent HPLC methanol was used with the stream of liquid N2 until it reached nearly 0.5 ml and then some mobile phase was added to reach 1ml. Then 20µl of the methanolic extract of the sample was injected into the HPLC column. The presence of each compound was determined by comparison of the peak area of the samples with that of the standard.

Mobile phase and solutions

1. The mobile phase prepared with a mixture of methanol: water (70:30) was used for the HPLC analysis of methanolic extracts of the untreated, crude effluent and biotreated plants.
2. The mobile phase with a binary mixture of acetonitrile: water (60:40) was used for crude effluent and biotreated silk dyeing industrial effluent.
3. The mobile phase for standards such as pigments, alkaloid caffeine and monosaccharides were prepared with 20% methanol and 0.2% phosphoric acid, methanol: water (80:20) and acetonitrile: water (90:10) respectively.

RESULT AND DISCUSSION

Analysis of the methanolic extracts of selected GLVs grown in fresh water in bio-nano UV visible spectrophotometer

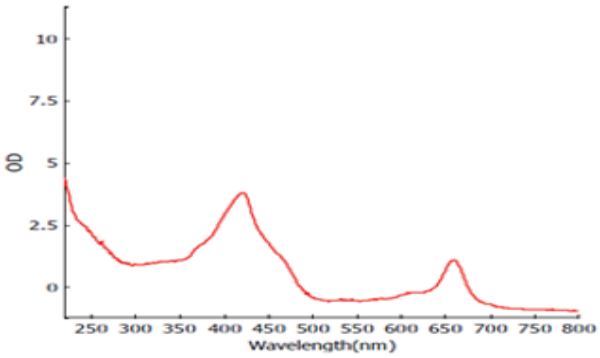

Figure 1: UV visible spectra of the selected GLV

Figure 1 depicts the spectra of methanolic extracts of selected GLV grown in freshwater subjected to bio-nano UV visible spectrophotometer. The intestines (Gramza A *et al.,* 2005).

B.juncea

The methanolic extracts of the GLVs such as *B.juncea* were subjected to UV visible spectrophotometer at a different wavelength from 250-800nm. The visible range of 400-700nm was taken into consideration. The methanolic extract of *B.juncea* has another peak in between 400-450 nm and 650nm. So the 450nm peak wavelength is selected for the analysis of differently treated samples to FTIR and HPLC.

Figure 2 shows the spectrum of dry powder of B.juncea grown in freshwater subjected to FT-IR (Fourier Transform Infrared Spectroscopy -IR affinity).

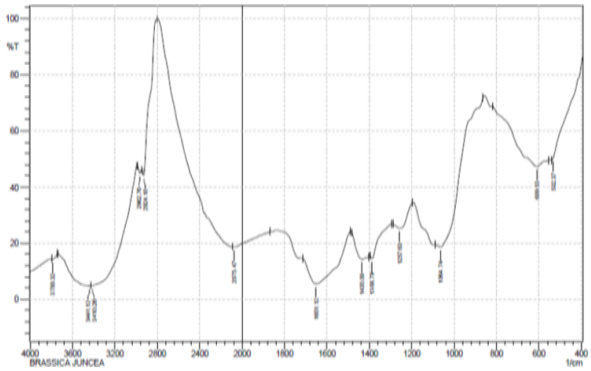

Figure 2: FT-IR spectrum of B.juncea grown in fresh water

The FT-IR spectrum contains the following functional groups detected in 3441, 3410wavenumber cm^{-1} has a strong, broad intensity which represents alcohol group with H-bonded stretch. The small sharp cluster of peaks at 2962, 2924 wavenumber cm^{-1} indicates the alkanes with C-H stretch. The 2075 wavenumber cm^{-1} corresponds to isocyanide (R—N═C═S). The medium peak of 1651 wavenumber cm^{-1} with –C≡C- stretch was identified as alkenes. The wavenumber 1435 cm^{-1} has a C-C stretch of an aromatic group and with variable bending in 1388 wavenumber cm^{-1} represents an alkane group. The 1257 wavenumber cm^{-1} with C-N stretch of small peak shows the presence of amine group presence. The C-O stretch of 1067 wavenumber cm^{-1} indicates alcohol group.

Similarly FTIR spectra of new cellulose paper show absorbance peaks located close to 3441 cm^{-1} that represents an O–H functional group, that represent a C–H functional group, and located close to 1050 cm^{-1} that represents a C–O functional group investigated of Transformer Paper in Mineral Oil-Paper Composite Insulation under Accelerated Thermal Aging (Abi Munajad *et al.,* 2018) and also peaks located at 1053 cm^{-1} closely to the observed peaks indicates presences of amines investigated in the mustard seeds. (Aparna Khanna *et al*., 2014).

The wavenumber 609 cm^{-1} has C-H bend corresponds to alkynes. The 532 wavenumber cm^{-1} with C-Br stretch belongs to alkyl halide.

Figure 3 illustrates the spectrum of dry powder of *B.juncea* grown in crude silk dyeing effluent subjected to FT-IR.

The FT-IR spectrum comprises 3417 wavenumber cm^{-1} with a strong and broad peak indicates the O-

H bond of alcohol/phenols. Similar to the previous spectrum 2924, 2854 wavenumber cm^{-1} with -C-H- stretch corresponds to an alkane. 2376, 2330 Wavenumber cm^{-1} did not match with the FT-IR standard chart and are not detectable. The 2167 wavenumber $cm^{-1}$with –C≡C- stretch shows alkyne group. The C=C Stretch of the conjugated double bond with 1627 wavenumber cm^{-1} corresponds to alkenes. The characteristic absorbance bands at 1435, 910 and 532 wavenumber cm^{-1} represent aromatics (-C=C- stretch), primary/secondary amine, alkyl halide respectively. 1249, 1033 wavenumber cm^{-1} corresponds to the aliphatic amine (-C-N- Stretch).

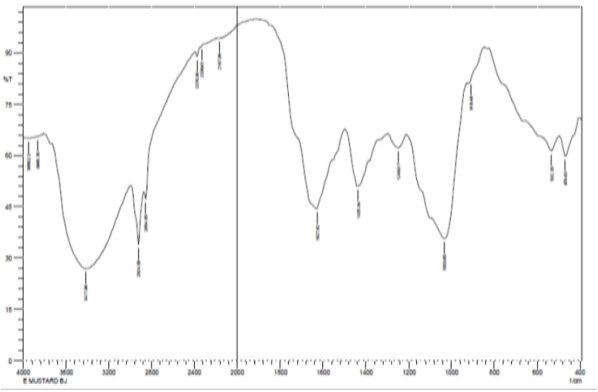

Figure 3: FT-IR spectrum of B.juncea grown in crude effluent

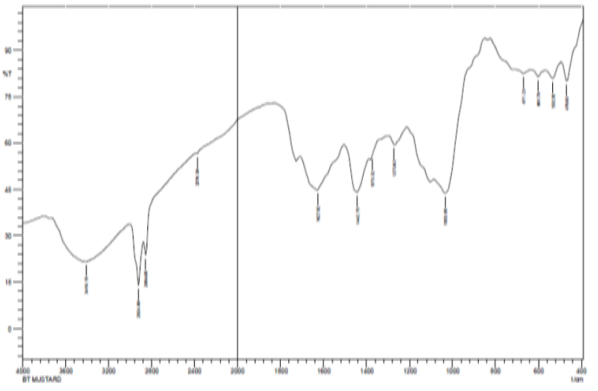

Figure 4: FT-IR spectrum of B.juncea grown in biotreated effluent

Figure 4 reveals the spectrum of dry powder of *B.juncea* grown in biotreated effluent subjected to FT-IR.

The spectrum with some peaks which has similarity in the wavenumber 3410, 2924, 1627, 532 cm^{-1} signifies alcohol/phenols (OH Stretch, H bonded), an alkane (C-H- Stretch), alkene, alkyl halide similar to the spectrum of *B.juncea* grown in the effluent. The other wavenumber cm^{-1} at 1442 belongs to aromatics (-C-C- Stretch). The small peaks at 671 and 601 wavenumbercm^{-1}corresponds to alkyl halide group with –C-Cl- stretch and –C-Br- stretch respectively.

Figure 5 shows the spectrum of dry powder of selected dyes (Direct 2y2, Direct yellow 5gll, Procell pineapple) subjected to FT-IR.

The dye powder subjected to FT-IR analysis confirms the presence of alcohol, alkane, alkynes, aldehyde, nitro groups, aliphatic amines, primary and secondary amine, an aromatic group and alkyl halide groups.

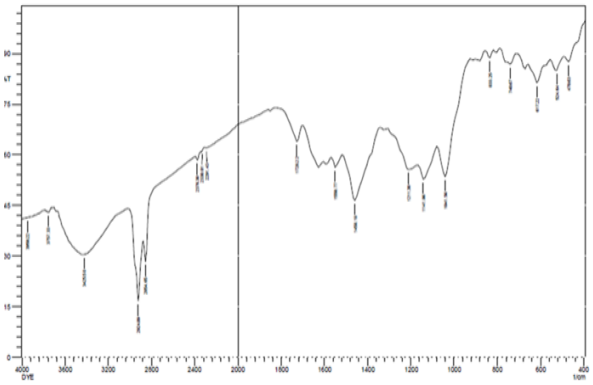

Figure 5: FT-IR spectrum of the selected dyes of silk dyeing effluent

Functional groups detection from the FT-IR spectrum of selected GLVs

Table 1 depicts the analysis of functional groups in the selected GLV of different treatments.

Table 1: Functional groups detected in the FT-IR spectra of selected GLV in different treatments

Functional Group	*B.juncea*		
	BJN	BJE	BJT
Alcohol	+	+	+
Alkane	+	+	+
Alkene	+	+	+
Alkynes	-	+	-
Carboxylic acid	-	-	-
Esters	-	-	-
Ethers	-	-	-
Isocyanide	+	-	-
Phosphine	-	-	-
Aromatic	+	+	+
Nitro groups	-	-	-
Amide	-	-	-
Aldehyde	-	-	-
Amine	+	+	+
Alkyl halide	+	+	+

Thus from the functional group studies by FT-IR, the alcohol, alkane, alkenes, aromatic, alkyl halides and amines were found in GLV irrespective of the treatments, even in crude effluent, the plants managed to synthesize these organic compounds. It means that this compound synthesis is not affected by the silk dyeing effluent. The alkynes were synthesized by the effluent treated plants which mean that effluent has induced the synthesis of alkynes. The amide group, amide, Nitro groups, esters,

ethers and carboxylic acid was completely absent in methanolic extracts of all the treatments. The isocyanide group was found only in *B.juncea*, grown in fresh water, which was unable to synthesize isocyanide group in plants grown in crude effluent and biotreated effluent.

HPLC analysis of the methanolic extracts of the selected GLVs grown in different treatments, untreated and biotreated silk dyeing effluent

Figure 6 reveals the chromatogram of a methanolic extract of *B.juncea* grown in fresh water, crude silk dyeing effluent and biotreated effluent which was subjected to High-Pressure Liquid Chromatography (HPLC) analysis.

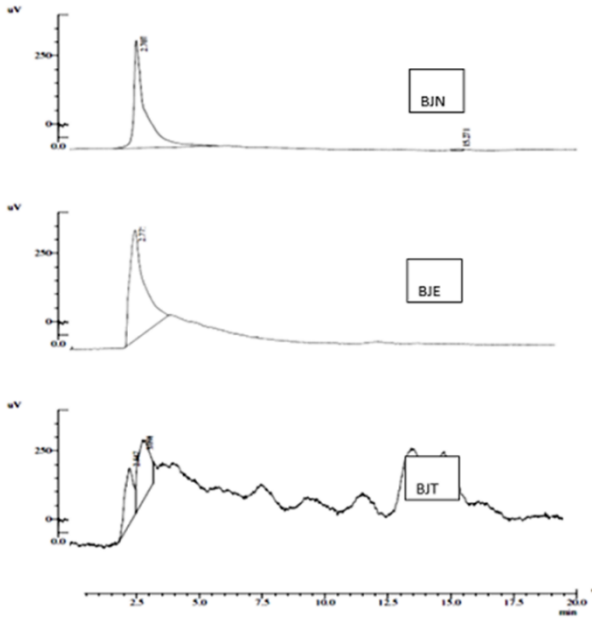

Figure 6: HPLC of B.juncea grown in fresh water, crude effluent and biotreated effluent; BJN: *Brassica juncea*, were grown in fresh water. BJE: *Brassica juncea* were grown in crude effluent: BJT: *Brassica juncea* were grown in biotreated effluent.

Figure 6 shows the HPLC of different methanolic extracts of *B.juncea*. The *B.juncea* plant (BJN) grown in freshwater revealed about 2 peaks of retention time 2.7 and 15.2 minutes which had a broad and small peak respectively while the methanolic extracts of *B.juncea* (BJE) grown in crude effluent had shown one broad peak of retention time (tR) 2.7 minutes. The loss of a peak may be due to the effect of effluent that might have hindered the synthesis of the compound while the biotreated *B.juncea* (BJT) had shown two peaks of retention time (tR) 2.5 and 3.0 minutes respectively. This assures that the biofertilizers have treated the silk dyeing crude effluent efficiently and reverted back its effect to freshwater methanolic extracts of *B.juncea.*

CONCLUSION

Thus from this study with spectroscopic and chromatographic analysis, the compounds were analysed with different treatments, and the effect of the silk dyeing effluent was seen its impact in the presence and absence of the functional group. It can be recommended that the biofertilizer treated crude silk dyeing effluent can be given to the environment which will not affect the functional groups of the GLV *Brassica juncea.*

Acknowledgements

We are thankful to the research scholars of the Department of Biotechnology during sampling at the disposal sites, which was a contaminated site with Silk dyeing effluent. I am thankful to Dr Sivagami Srinivasan for her fullest support. We also thank anonymous reviewers for their comments to improve the manuscript.

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