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Research Article

SUSTAINABLE TECHNOLOGY FOR RECALCITRANT AZO DYES BY FUNGAL SPECIES ISOLATED FROM INDUSTRIAL EFFLUENT

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ABSTRACT

Synthetic dyes have a repugnant effect on the aquatic ecosystem and their venomous components have to be removed from the effluent before their exemption. The decolourization of synthetic dyes by fungi is emerging as an effective and clean technology. Fungal-abetted processes are amongst the finest approaches for transformation, degradation and decolourization of recalcitrant dyes. This study was carried out to find out the qualities of textile effluents with the estimation of physicochemical parameters of textile effluents, isolation and identification of fungi. The physicochemical parameters were demarcatedas per the standards prescribed by CPCB.15 fungi isolated from textile effluent and effluent-contaminated soil samples were evaluated to decolourize textile dyes - Reactive Red ME-4B and Brill blue-R. 9 different fungal species were further identified as *Aspergillus spp*, *Penicillium spp, Mucor, Curvularia spp, Helmintho sporium spp, Fusarium spp, Trichoderma spp, Rhizopus, spp* and *Alternaria spp* by 18S rRNA Sequencing. These 9 isolates with potential decolourization were assessed via primary and secondary decolourization. The study also showed that *Penicillium spp*, *Aspergillus spp* and *Trichoderma spp* are highly efficient in decolorizing (85.41, 98.80and83.23 %) in 6 days as used in the present investigation. The results have demonstrated the potential of the fungal isolates for the treatment of dyes contaminated textile effluent.

Keywords: Physicochemical parameters, Fungal isolation, 18S rRNA sequencing, Decolourization, Recalcitrant dyes.

1. INTRODUCTION

Growing environmental pollution resulting from rapid industrial development is one of the challenges facing the modern world. The textile industry releases about 10 to 15% of the dye, which find its way into waste water. It chiefly consists of residue dyes, surfactants, salts, and chlorinated compound [1]. Thus, the colour removal of textile waste water is a major environmental concern. Therefore, industrial effluents like textile waste water containing dyes must be treated before their discharge into the environment. The dye waste water from the textile is one of the most difficult waste water to treat, because of their commercial importance. The impact and toxicity of dyes have been extensively studied. Colour can be removed from wastewater by chemical and physical methods including absorption, coagulation, flocculation, oxidation and electrochemical methods [2]. The chemical and physical methods used may also include ion exchange, irradiation, precipitation, ozonation, oxidation via chlorine, electro-floatation, electrolysis, and peroxide [3]. These methods are quite expensive, have operational problems and generate huge quantities of sludge. Among low-cost, viable alternatives for effluent treatment and decolourization, the biological systems are recognized by their capacity to reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by convectional aerobic degradation. The treatment based on using microbes capable of degrading or decolourizing these recalcitrant compounds is environmental friendly and can lead to mineralization of the target compounds. The effectiveness of these treatment systems depends upon the survival and adaptability of the microorganisms during the treatment processes [4]. Fungi have shown strong adaptability and efficiency in the removal of these aromatic compounds. In this context, fungi offer an efficient system due to their large surface area and easy solid–liquid separation [5]. Fungi also possess multiple mechanisms for degradation and biotransformation of organic and inorganic contaminants. The fungal mycelia have an additive

advantage over single-cell organisms by solubilizing the insoluble substrates by producing extracellular enzymes. Due to an increased cell-to-surface ratio, fungi have greater physical and enzymatic contact with the environment. The extra-cellular nature of the fungal enzyme is also advantageous in tolerating high concentrations of the toxicants. Many genera of fungi have been employed for dye decolourization either in living or dead form [6]. The current study aimed to explore and compare the decolorization efficiency of two different textile azo dyes by 9 different fungal species isolated from the effluent of textile effluent-contaminated soil.

2. MATERIAL AND METHODS

2.1. Media and chemicals

The textile dyes (Red ME-4B and Brill blue R) used for the present investigation's decolourization were gifted from Colourtex, Surat (Gujarat). All media components and chemicals used in the present study were of analytical grade and purchased from HiMedia Laboratories (Mumbai, India).

2.2. Sampling

Soil samples and effluent samples from different areas of Surat, Vapi and Ankleshwar, Gujarat state, were collected in sterile polypropylene bags and glass bottles respectively. The soil samples were kept in a refrigerator at 4°C until the fungi were isolated. Effluent samples were immediately processed for physicochemical analysis [7].

2.3. Physico-chemical analysis of Textile effluent

The physicochemical parameters of the effluent such as colour, pH and total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), chlorides, total solids (TS), electrical conductivity (EC), turbidity, alkalinity and acidity were determined as per the standard prescribed by CPCB [8]. The Physico-chemical analysis of the samples was carried out in the laboratory as per the Standard Methods of APHA 23rd [9].

2.4. Isolation and identification of dye-decolourization fungi

The Potato Dextrose Agar (PDA) was used in the isolation of fungi present in the textile effluent contaminated soil, a standard dilution plate method was adopted, and inoculated PDA plates were incubated at 30°C temperature for 3-5 days. After incubation, colonies were isolated and identified by LPCB staining [10].

2.5. Identification of the fungal isolates

The fungal isolates were identified by routine mycological methods, i.e., Lactophenol cotton blue staining and plating on potato dextrose agar medium.

2.6. Maintenance of fungal isolates

Well-grown fungal colonies were maintained on Rose Bengal agar slants and stored at 4°C.

2.7. Molecular characterization of fungal isolate

Molecular identification of fungal isolate was carried out by using the standard Neighbor-joining method for genomic DNA isolation. Universal primers of ITS region ITS1 and ITS4 were amplified by PCR. DNA sequencing reaction of PCR amplicon was carried out with ITS1/ITS4 primer using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The gene sequence was used to carry out BLAST with the database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software programs [11].

2.8. Decolourization studies on solid media

The solid media were prepared with PDA medium and the addition of each dye to a total concentration of 50mg/L. A mycelium plug derived from the edge of fungal strains grown on PDA medium plates for 4 days at 30°C was transferred to the centre of a dye containing solid medium plate and inoculated at 30°C for 1 week. The formation of decolourized zones under or around the developing mycelia was monitored for dye decolourization. All the agar plate assays were performed in triplicate [12].

2.9. Decolourization analyses

Erlenmeyer flasks (250ml) containing 100 ml of decolourization medium containing (g/L) glucose 1.0, $MgSO_4.7H_2O$.2.0, KH_2PO_4 1.5, CaCl1.5, NH_4Cl 0.15 were autoclaved at 121 °C for 15 min, cooled and then inoculated with 3 agar plugs of size 6 mm diameter punched from the leading edge of pre-grown fungal culture on PDA plates and 1000 ppm sterilized dye was added to the flasks and incubated for 10 days at 30 °C. Uninoculated nutrient medium served as control [13]. Dye decolourization was determined

spectrophotometrically by monitoring the absorbance of samples at k max of the respective dyes using a UV-Visible spectrophotometer (Shimadzu UV 1800, Japan). Results are reported as the mean amount of decolourization for three replicates. The decolourization expressed in % of initial dye concentration was calculated as follows:

$$D= (A_0-A_1/ A_0) \times 100$$

Where, D= Decolourization rate, A_0= Initial absorbance or absorbance of control, A_1= final absorbance or the absorbance of the samples test.

Table 1: Analysis of Physico-chemical parameters of untreated textile effluent

Parameters	S1	S2	S3	S4	CPCB
pH	8.00	4.70	6.74	6.8	5.5-9.0
TDS	400mg/l	350mg/l	2223mg/l	2300mg/l	2100mg/l
TSS	1300mg/l	450mg/l	800mg/l	970mg/l	100mg/l
TS	1700mg/l	800mg/l	3023mg/l	3207mg/l	-
BOD	177mg/l	50mg/l	78mg/l	95mg/l	30mg/l
COD	600mg/l	1300mg/l	772mg/l	1096.7mg/l	250mg/l
DO	0.6	-	-	-	-
Conductivity	858µs/cm	779µs/cm	1.368mS/cm	4.33mS/cm	-
Turbidity	16NTU	38NTU	130NTU	150NTU	10 NTU
Acidity	120mg/l	250mg/l	350mg/l	390mg/l	-
Alkalinity	200mg/l	150mg/l	840mg/l	580mg/l	600mg/l

3. MATERIAL AND METHODS

3.1. Physico-chemical analysis of textile effluent

In general, the textile industry releases ample pollutants from all stages in the processing of fibers and fabrics. Amongst various industries, Central Pollution Control Board has listed the dye and dye intermediates industry as one of the profoundly polluting industries. The physicochemical parameters of textile dye effluent were studied to understand the characteristics of the effluent, various hazardous chemicals used during the process and its effects on natural systems when it is discarded.

The result of the study shows that it is alkaline and acidic with high BOD, COD, organic particulate matter, and blackish colour. An unpleasant may be due to microbial growth or H_2S gas. The pH of the textile effluent was alkaline at about 8.0 and acidic pH at 4.70, 6.74 and 6.8. The total dissolved solid was found to be 400, 350, 2223 and 2300 mg/ml. The total suspended solids were found to be 1300, 450, 800 and 970mg/ml. The value of BOD was found to be177, 50, 78 and 95 mg/l and COD was found to be 600, 1300, 772 and 1096.7 mg/l and other parameters as shown in Table 1.The alkaline pH of the effluents is due to the use of carbonate, bicarbonate, H_2O_2 and NaOH during the bleaching process in the textile industry. The acidic pH of effluents affects the physicochemical properties of water which in turn adversely affects aquatic life, plants and humans. This also changes soil permeability which results in polluting underground resources of water [14]. An increase in COD can be due to a huge number of industrial wastes such as detergents, softeners, formaldehyde-based dye fixing agents etc. Textile industries use organic substances as raw materials and high levels of dissolved organic matter consume large amounts of oxygen and increase BOD level, which undergoes an aerobic fermentation process leading to formation of ammonia and organic acids. Hydrolysis of these acidic materials causes a decrease in water pH values. An increase in BOD may cause hypoxia conditions with consequent adverse effects on aquatic biota [15].

3.2. Isolation and identification of dye-decolorizing fungi

Dye-decolorizing fungi have been frequently isolated from textile effluents and soils exposed to dye wastes. In the present investigation, soil samples collected from waste disposal sites of three different textile industries were screened for the occurrence of fungi.

Fungal species native to the sampling sites were isolated on PDA medium using dilution plate method. The species were identified by their morphological characteristics based on Lactophenol cotton blue staining and colony morphology on Potato dextrose agar [16].

A total of 15 fungi were isolated and identified (Table 2). Out of these, 9 species are *Aspergillus spp*, *Penicillium spp, Mucor, Curvularia spp, Helmintho sporiums pp, Fusarium spp, Trichoderma spp, Rhizopus spp* and *Alternaria spp*. were ubiquitous and recovered from all the screened soil samples. The incidence of fungi in polluted water and soil depends on the availability of nutrients, oxygen and biological, physical and chemical features of the pollutant. The isolated strain was analysing the 18S rRNA sequence for identification of the isolated microorganism. Based on ITS analysis, isolate P94 is assigned as *Aspergillus quadrilineatus*.

Table 2: Fungal strains identified in different industrially polluted soil

Organisms	Sample 1	Sample 2	Sample 3	Sample 4
Aspergillus sp	+	+	+	+
Penicilliumsp	+	+	-	+
Mucor	-	+	-	-
Alternaria sp	+	-	-	-
Curvulariasp	-	-	-	+
Helminthosporiumsp	-	+	-	-
Fusarium sp	-	-	+	-
Trichoderma sp	-	+	-	-
Rhizopus sp	+	+	-	-

Unit; + = Present, - = Absent

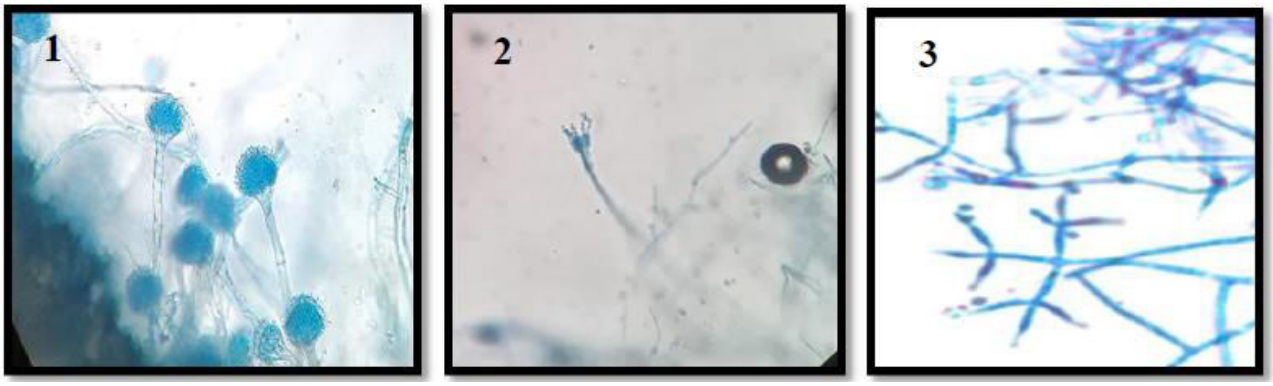

Fig. 1: Microscopic view of fungi: 1. *Aspergillus spp,* 2. *Penicilliumspp* 3.*Trichoderma ssp*

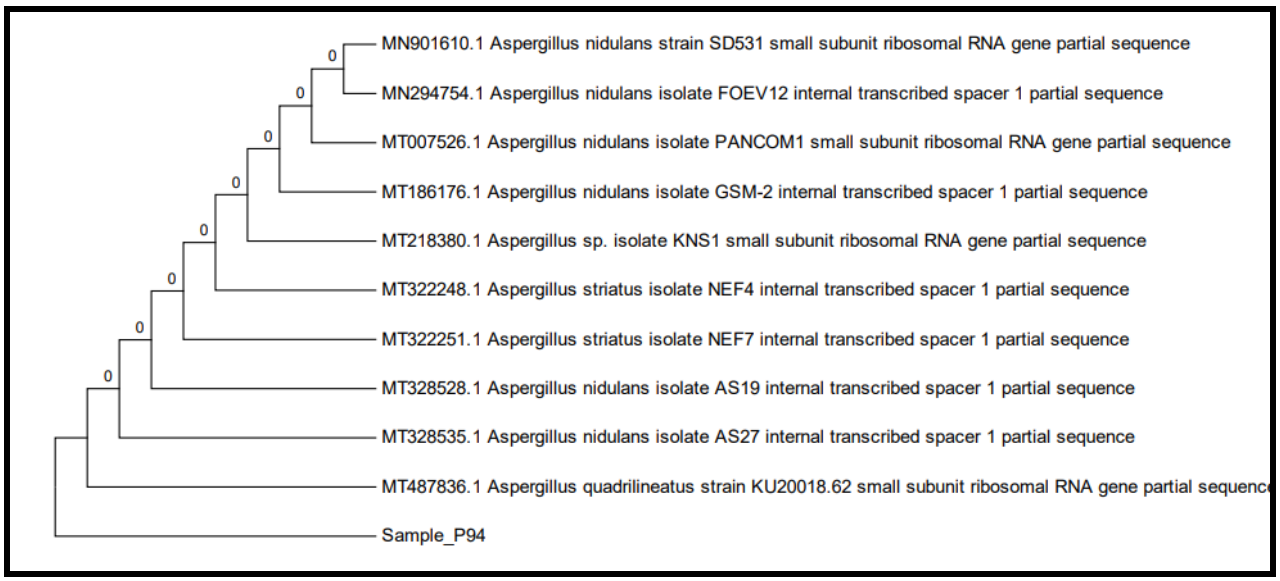

Fig. 2: Phylogenetic analysis of selected fungus as *Aspergillus quadrilineatus* P94

3.3. Degradation studies on solid media

The decolourization capability of 9 fungal strains was assessed on dye-containing PDA plates with 2 types of synthetic dyes inoculated at 30°C for 1 week. Nine fungal strains that caused either a partial or full decolourization were screened out of 15 strains of freshwater fungi isolated from the decayed wood samples. The dye decolourization results on agar plates showed that these fungal strains had the ability to decompose 2 types of dyes. Likewise, different fungi had different dye decolourization abilities. For example, strain *Aspergillus spp* had the capability to decompose all 2 different types of dyes tested in these experiments (Fig. 1 & 2).

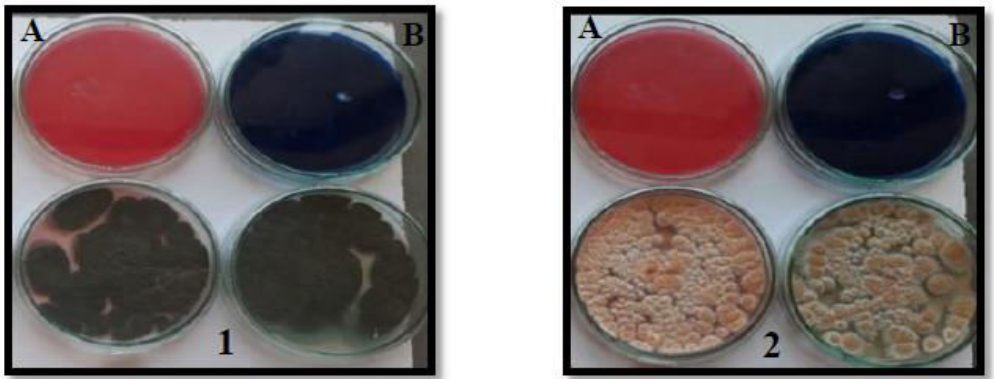

Fig. 3: Decolorization of 6 dyes by *Penicilliumspp* and (2)*Aspergillus spp* fungal on PDA medium that contained 50mg/L of different dyes inoculated during 7 days. (A) Red ME-4B(B) Brill Blue R

3.4. Decolourization analyses

In the present investigation, all nine isolated fungal species were used to study decolourization of textile dyes viz., Red ME-4B and Brill Blue R at 1000 mg/L in a decolourization medium. The decolourization efficiencies were measured after 10 days of incubation under controlled conditions. The results revealed that all the isolated fungal species had variable potential to decolourize different textile dyes (Table 3).

Table 3: Decolorization rates (%) of 2 dyes after the treatment with 9 fungal strains for 10 days in liquid media

Decolourization (%)		
Organism	**Red ME-4B**	**Brill Blue R**
Aspergillus spp	98.8 ±0.1	90.1 ±0.1
Penicillium spp	85.41 ±0.08	88.133 ±0.1
Mucor	71.16 ±0.15	68.27 ±0.1
Alternaria spp	82.33 ±0.15	79.54 ±0.01
Curvulariaspp	78.9 ±0.1	73.23 ±0.2
Helminthosporiumspp	80.26 ±0.20	82.13 ±0.05
Fusarium spp	62.36 ±0.01	59.2 ±0.00
GGTrichodermaspp	83.23 ±0.05	82.33 ±0.01
Rhizopus spp	59.33 ±0.15	62.7 ±0.1

The most potent fungal species in decolourizing Red ME-4B was *Aspergillus spp* followed by *Penicillium spp*, *Mucor*, *Curvularia spp, Helmintho sporium spp, Fusarium spp, Trichoderma spp*, *Rhizopus spp*, *Alternaria spp*. Brill Blue R was most efficiently decolourizing by *Aspergillus spp* followed by *Penicillium spp*, *Curvularia spp*, *Helminthosporium spp*, *Fusarium spp*, *Rhizopus spp, Alternaria spp*. Red ME-4B was the most recalcitrant among all the dyes studied for decolourization by the isolated fungi. The results also showed that Red ME-4B dye was highly degradable by all the fungal species and had a decolourization efficiency of 98.80% (Table 3). Similarly, Red M8B was degraded to 85.41% by *Penicillium spp*. Degradation kinetics of the fungal species revealed that maximum degradation was achieved after 10 days of incubation and further incubation had no effect on the degradation of dyes.

4. DISCUSSION

The presence of dyes in industrial products currently results in wastewater pollution, and these pollutants need to be treated before they are discharged. The ability of microorganisms to perform dye degradation has received considerable attention. The fungal isolates in this research provide a new avenue for approaching the biodegradation of dyes in the future. The fungi used for dye biodegradation were mainly isolated from forest ecosystems. The isolates of fungi used to decolourize water conditions will probably have the efficacy of bioremediation of dyes because these fungi have the

ability to secrete enzymes that contribute to carbon recycling in liquid environments [17]. The variation and biodiversity of the isolated fungi from different geographical locations show different factors that affect the growth and distribution of fungi; these factors include soil pH, moisture content, salinity, organic carbon, nitrogen sulfur and potassium [18]. The microscopic examination of the shape of the spore-producing structures was used for further identification. The morphological examination and identification of fungi are useful for the identification of isolates up to the family or genus level [19]. In the present study, 9 fungi showed high efficiency for the decolourization of Red ME-4B and Brill Blue R dyes. The molecular identification was carried out by DNA barcoding using the ITS region sequencing. The ITS rDNA sequences were compared to those in the databases using NCBI-BLAST. In the present investigation, textile effluents from different areas of Surat, Vapi, and Ankleshwar, Gujarat state were highly coloured and had BOD, COD and pH respectively. These results are in agreement with earlier reports from textile effluents (Devi and Kaushik 2005). High pH is mainly due to the use of carbonate, bicarbonate, H2O2 and NaOH during the bleaching of the textile. nine fungal species were successfully identified using taxonomic guides and standard procedures. TDS mainly consists of inorganic salts such as carbonates, bicarbonates, Chlorides Sulphates, phosphates, Nitrates, Calcium, Magnesium, Sodium and Potassium, Iron etc. Dissolved salts in water cause skin dehydration in animals and give a laxative effect and unpleasant mineral taste to water [20]. The high value of TSS increases sedimentation rates and turbidity levels of water, thereby influencing the oxygen demand and reducing photosynthesis. It also increases the levels of pathogens and contaminants, thus distressing the food chain of aquatic biota [21]. Textile industries use organic substances as raw materials and high levels of dissolved organic matter consume large amounts of oxygen and increase BOD level, which undergoes an aerobic fermentation process leading to formation of ammonia and organic acids. Hydrolysis of these acidic materials causes a decrease of water pH values. Increase in BOD which may cause hypoxia conditions with consequent adverse effects on aquatic biota [22]. Increase in COD can be due to a huge number of industrial wastes such as detergents, softeners, formaldehyde-based dye fixing agents etc. A higher concentration of COD in water implies toxic conditions and the presence of biologically resistant organic substances. Hence the effluent is incompatible with the survival of water-living organisms due to the reduction of DO content. *Aspergillus quadrilineatus, Penicillium spp* and *Alternaria spp*. were the most important species and recovered from all the sites studied (Table 3). From the results of the present study, it is also clear that these species were also found to be efficient degraders of textile dyes. Most of the isolated fungal species were able to decolourize textile dyes (1000 mg-L) within 10 days under static culture conditions. In our study, it was observed that fungal isolates were able efficiently to degrade a dye.

5. CONCLUSION

In this study, *Aspergillus quadrilineatus* (P94) isolated from the textile effluent-contaminated soil has a high potentiality in azo dye decolourization. The findings have been found to be an efficient decolourization of azo dyes may be attributed to either the action of enzymes present in *Aspergillus quadrilineatus* or by biosorption mechanism for the treatment of azo dyes. Experiments are planned to further examine the pathways and enzymes involved in the degradation of these dyes. Application of traditional wastewater treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using fungi can be applied for fine-tuning of wastewater treatment. Biotreatment offers an easy, cheaper and effective alternative for the colour removal of textile dyes.

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Conflict of interest

None declared

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7. REFERENCES

1. Selvam K, Swaminathan K, Chae KS. *Bioresource Technology*, 2003 Jun **1; 88(2)**:115-119.
2. Bibi I, Javed S, Ata S, Majid F, Kamal S, Sultan M, et al. *Biocatalysis and Agricultural Biotechnology*, 2019 Nov **1; 22**:101420.
3. Nosheen S, Nawaz H, Rehman KU. *Int J Agric Biol.*, 2000; **2(3):**232-233.

4. Mishra A, Malik A. *Bioresource technology*, 2014 Nov 1; **171**:217-226.
5. Kaushik P, Malik A. *Journal of Scientific & Industrial Research*, 2009; **68**:325-331.
6. Alsohaili SA, Bani-Hasan BM. *Jordan Journal of Biological Sciences*, 2018 Sep 1; **11(3)**.
7. Ben AB, Amutha E, Pushpalaksmi E, Jenson SJ, Annadurai G. *Journal of Applied Sciences and Environmental Management*, 2020 Aug 7; **24(7)**:1203-1208.
8. Jairajpuri M, Patel K, Parekh H. *Journal of World Medica Research & Review*, 2016; **Vol 2 (1)**.
9. Gaddeyya G, Niharika PS, Bharathi P, Kumar PR. *Advances in Applied Science Research*, 2012; **3(4)**:2020-2026.
10. Bao Z, Ikunaga Y, Matsushita Y, Morimoto S, Takada-Hoshino Y, Okada H, et al. *Microbes and environments*, 2012; **27(1):**72-79.
11. Aneja KR. Experiments in microbiology, plant pathology and biotechnology. New Age International; 2007.
12. Yang P, Shi W, Wang H, Liu H. *Brazilian journal of microbiology*, 2016 Oct; **47**:828-834.
13. Rosales E, Pazos M, Sanromán MA. *Desalination*, 2011 Sep 1; **278(1-3):**312-317.
14. Nirgude NT, Shukla S, Venkatachalam A. *Rasayan Journal of Chemistry*, 2013; **6(1):**68-72.
15. Manikandan P, Palanisamy PN, Baskar R, Sivakumar P, Sakthisharmila P. *International Journal of Advance Research in Science and Engineering*, 2015 Jan 1; **4(2):**93-104.
16. Lee JW, Gwak KS, Park JY, Park MJ, Choi DH, Kwon M, Choi IG. *The Journal of Microbiology*, 2007 Dec; **45(6):**485-491.
17. Ponraj M, Gokila K, Zambare V. *International journal of advanced biotechnology and research*, 2011; **2(1):**168-177.
18. Madhav S, Raju NJ, Ahamad A, Singh AK, Ram P, Gossel W. *Environmental Earth Sciences*, 2021 Sep; **80(17)**:585.
19. Sharma MS, Raju NS. *International journal of environmental sciences*, 2013; **3(5)**:1569-1576.
20. Devi M, Kaushik BD. *Indian Journal of Microbiology*, 2005; **45(1):**41.
21. Shah I, Sudarsan JS, Shah U, Ramesh S, Sehran M. *InAIP Conference Proceedings,* 2019 Jun 21; **(Vol. 2112, No. 1)**: AIP Publishing.
22. Chockalingam N, Banerjee S, Muruhan S. *Environment and Natural Resources Journal*, 2019 Jan 28; **17(2)**:41-53.