Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [1] January 2023: 317-324.

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Bioremediation of Azo dyes by microbial consortia isolated from textile effluent contaminated soil.

Dhangar U. S[1*], Shinde S. R[1], Patil N. N[1], Ghule A.V[1], and Moundekar A. A.[1].
1Department of Microbiology, Faculty of Science, PDEA'S Annasaheb Magar Mahavidyalaya, Hadapsar, Pune 4112028, State-Maharashtra, India.
**Corresponding author: hanshulganeshdhangar@gmail.com*

ABSTRACT:
Congo red (CR) and Methylene blue (MB) are one of the best known azo dyes which has azo bond (-N=N-) hard to break. They are commonly use as indicator dyes of Azo dyes in textile industries. The current work scrutinized the de colorization of these azo dyes in distilled water using microbial consortia. The fungal and bacterial isolates were screened for de colorization of MB and CR at different concentrations. Cultural, Morphological and Biochemical characterization of isolates shows the presence of Aspergillusspp. and Acinetobacter spp. Acinetobacter spp. able to decolorize Congo red 42.3% and Methylene blue 45.41% at 0.5% concentration of respective dyes. Aspergillus spp. decolorized 59.53% Congo red and 38.00% methylene blue at 0.5% concentration of respective dyes. UV-Spectroscopy and FTIR analysis of samples before and after growth of Aspergillus spp.at respective concentration of MB shows de colorization of MB is by absorption of MB dye on the surface of Aspergillus spp. The overall study showed de colorization of MB and CR dye present as indicator dye in textile effluent by simple microbial absorption method. This method is found more applicable for removal of toxic dyes from textile effluent. Phytotoxicity of the dye solution resulting from this treatment on Zeamaizeshows lower toxic nature compared to untreated solution of the respective dyes.
***Key words**: Methylene Blue (MB), Congo red(CR), Aspergillus spp., Bio-absorption, Azo dye, Acinetobacter spp.*

Received 02.11.2022 Revised 23.11.2022 Accepted 10.12.2022

1. INTRODUCTION:

1.1 Review of literature:

Azo dyes are widely used in textile, paper and pulp industries. Azo dyes produce bright, high-intensity colors, have fair to good stronghold properties, are economical to produce and account for more than half of all commercial dyes used. Use of Azo dyes are increasing this is in turn would increase wastewater generation from dyeing industrial activities [1].Dye waste water is usually characterized by high COD,BOD,TSS,TDS as lots of synthetic dyes, bases, acids, salts, oxidants, reductants, many chemical substances and metal ions.[2]. Azo dye contain antimicrobial activity was discovered by Gerhardt Domagk. Azo reduction can be accomplished by skin micro flora, human intestinal micro flora, environmental microorganisms, to a lesser extent by human liver azo reductase, and by non-biological means. [3].

Azo dyes do not degrade under natural environmental conditions. When the waste water has been released from industry, it wills bio-accumulate in the environment, released poisoning issues not only in the water, but also affecting the entireness of the ecosystem. Azo dyes which are banned by the European Commission. [4]. Incompetence in dying process, poor hold of spent effluent and inadequate treatment of wastes from dyestuff industries lead to dye pollution in soil and natural water bodies [18].There should be economical and eco-friendly method that produces a considerably lesser amount of intermediate toxic compounds. Use of different microorganisms like fungi, bacteria, yeast, algae for remediation of dyes from textile effluent by degradation, absorption and accumulation of dyes found more applicable. [5].

1.2 Effect of azo dye:

Synthetic azo dyes are widely used in industries. The antimicrobial effect of red azo dye Prontosil was caused product sulfanilamide by the reductively cleaved (Azo reduction). The significance of azo reduction is thus revealed. [6].

Azo reduction can be accomplished by skin micro flora, environmental microorganisms, and intestinal micro flora to a lesser extent by human liver azo reductase, and by non-biological means. [6].
However, the carcinogenicity of many azo dyes is due to their cleaved product such as benzidine and aromatic amine. Benzidine induces various human and animals allergic reactions, tumor formation and endocrine disruption. [6].
Many azo dyes and their reductively cleaved products such as benzidine as well as chemically related aromatic amines are reported to affect human health, causing allergies and other human maladies. [6].
After coloring the fabrics, 10–15% of used dyes get discharged into the textile effluent and then into aquatic ecosystem [7].To minimize the toxic effect of dye effluent for the reuse of water for irrigation of plants or directly to discharge treated water in fresh water without harming the environment.

2. MATERIALS AND METHODS:

2.1 Dyes and Chemicals: Two dyes were used for dye decolorization experiments:Methylene Blue (MB) and Congo red (CR). All the dyes used in present study were purchased from Loba chemie. Both the dyes were used for de colorization experiments in different concentrations. On the basis of primary study 0.1-0.5% of each dye were selected. [8]

2.2 Sample collection:

Textile effluent and soil sample from nearing textile industrial area was collected. Sample had been collected from the point where all types of pollutants will enter in the effluent.which is located in Ramtekadi, Hadapsar, Pune, Maharashtra 411013, India Lat 18.497094° Long 73.921217° date 15/03/2022 at 11:57 AM.

2.3 **Isolation of dye degrading microorganisms from effluent**:

The isolation of dye degrading microorganisms by diluting the collected effluent with saline water, and then serial dilution of the textile effluent and soil samplewas done. Serially diluted samples wereinoculated inde colorization broth (5-g glucose, 2.5-g yeast extract, and 2.5-g NaCl in a final volume of 500 ml)and incubated at 37^0 C for 24 hrs. After incubation suspension was used further for screening test for dye decolorization. [9]

2.4 **Screening test for dye degrading ability of microorganisms**:

Screening of textile dye degrading microorganisms from effluent and soil sample was done by using sterile Mineral salt mediumand Potato dextrose agar with particular concentration (0.5%) of Methylene blue and Congo reddye. Isolates can be selected on the basis size of clear zone around growth showing higher decolorizing potential. [9]

2.5 Identification of selected bacterial and fungal isolates:

Based on dye degrading ability selected bacterial and fungal isolates were identified by using cultural, morphological and biochemical characterization. For bacterial isolate Berge's manual of bacterial systematics and for fungal isolates fungal systematics by Lodder was used.

2.6 Dye decolorization assay:

Screened isolates were inoculated and incubated in respective broth with each dye at different concentration (0.1-0.5%). A negative control, dye solution without the inoculum was also kept for incubation at 30^0 C for 24 hrs. for bacterial isolates and 48 hrs. for fungal isolates. After incubation the sample were withdrawn at time intervaland checked for absorbance in UV-vis double beam Spectrophotometer at respective absorbance maxima for each dye. Samples were centrifuged at 10,000 rpm before taking absorbance. De colorization percentage was calculated by using formula: [9],[19].

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

2.7 **Bio -absorption Assay**:

The dye degradation by each isolates was confirmed through spectral analysis of colorimeter, UV-Vis spectroscopy, FTIR spectroscopy. [10].

a) UV-Visible Spectroscopy:

For the UV-Vis spectral analysis of dye de colorization, the decolorized solution wasscanned against a dye control and the peaks cross-matched uses a spectrophotometer [11]. The highest concentration of dye which completely decolorizedselected for this analysis against a dye control. The peaks obtained before and after decolorization by bacterial and fungal isolates were analyzed to study biabsorption ability [10].

b) Fourier Transform Infrared Spectroscopy (FTIR):

The functional groups of the absorbed dyes were analyzed with FTIR spectroscopy. Sample of MB and CR with and without isolates were checked for FTIR spectroscopy. After incubation the cell pellet was collected by centrifugation at 5000rpm for 10 min. Lyophilizedcell pellet used for FTIR analysis. [12].

2.8 **Phytotoxicity study:**

Treated synthetic waste effluent which having more amount of Methylene blue(MB) and Congo red(CR)in measurable amount was checked for plant toxicity by seed germination assay with negative control as a water not having dye in it and positive control as a water with Methyl Red (MR) in appropriate concentration. [13]

3. RESULTS AND DISCUSSION:

3.1 Screening and Identification of dye decolorizing isolates:

In the present study one fungal and one bacterial isolates were screened for de colorization of MB and CR at 0.5%. From the cultural, Morphological and Biochemical characterization isolates were identified at genus level. The fungi as *Aspergillus spp*. and bacteria as *Acinetobacter spp.* were identified.

3.2Dye de colorization assay:

Aspergillus spp. and *Acinetobacter Spp.*were used to de colorized of MB and CR at different concentration.The highest de colorization by both the isolates for respective dyes was seen at 0.1 %. Maximum concentration was found as 0.5% at which both isolates showing de colorization of respective the dyes. Differences in decolorization rate between Congo red and Methylene blue. *Aspergillus spp.* decolorized 59.53% Congo red and 38.00% Methylene blue.*Acinetobacter spp*. is also able to decolorize Congo red 42.3% and Methylene blue 45.41%.

3.3 FTIR analysis:

FTIR spectra of *Aspergillus spp*. shown in the spectral region 500-400 cm-1 alkyl halides C-I, and C- Br stretching mode halo compound are present. After treated Congo red and Methylene blue by *Aspergillus spp*. clearly reveals that for the major bands in the region 2158.00 cm-1 sp2 hybridization C–C stretch band and 465.26cm-1 stretching mode (C-I and C- Br.) FTIR Spectra of untreated *Acinetobacter spp*. is also shown in 500-400 cm-1 spectral region, stretching mode halo compound are present. Congo Red and Methylene Blue dye which shows absorption of the respective dyes on Acinetobacter spp. variation in bond suggested that the Aspergillus spp. and Acinetobacter spp. bio- absorption

DISCUSSION:

Acinetobacter spp. comparatively less decolorize than *Aspergillus spp*. *Aspergillus spp*. and *Acinetobacter spp*. Shown ability to de colorization of Methylene blue and Congo red dye which persist in environmental. The present study showed fungal and bacterial isolates absorbing the dyes may be they utilizedye as a carbon and energy source.De colorization of azo dye was assessed by calculating percentage of decolorization,UV spectroscopy and FTIR, Colorimetric method. Dye concentration was found to affect the dye decolorizationsrate (0.1% - 0.5%). Maximum wavelength of Congo red (λmax 460 nm) and methylene blue (λmax 620 nm) Using UV spectroscopy.The result of present study iscomparable to that of VeenaSreedharan. etal, 2021 who founded desirable bacterial and fungus such as *Acinetobacter* and *Aspergilus Spp*. ability to decolorized two azo dye.Congo red (464nm) and Methylene blue (620nm) has Maximum wavelength was confirmed by UV spectroscopy.We noted also that the isolated strain *Aspergillus* was more effective on Congo red (59.50%) and*Acinetobacter* was more effective on methylene blue (45.41%).Similar result was obtained with mixed culture which has been reported (61.53%) for Congo red and (47.70%) Methylene blue. CR and MB absorption was confirmed by FTIR. In order to elucidate the nature of functional groups responsible for the biosorption, FTIR analysis of the lyophilized biomass carried out before and after decolorization of dye by *AspergillusSpp*. and *Acinetobacter Spp.*More functional groups were found absorbed on the surface of the lyophilized culture after dye treatment.Phytotoxicity analysis revealed the toxicity of dyes on *zea maize* before and after treatment. Seed germination assay was carried out by using water agar with dye before and after treatment. Seed germination assay shown decreased toxicity level of dye after treatment, which can be concluded from relative germination rate in percentage.The present literature shown the CR and MB was successfully decolorized by *AspergillusSpp*and *Acinetobacter Spp*with high decolorizationefficiency (59. 53% and 45.51%) respectively at 0.5% of dyes.The increase in the rate of decolorization of Congo red and Methylene blue was identified by the microbial consortium47.7% and 61.53 %respectively.Mixed microbial consortia were found effective on textile dye degradation by Krishnamoorthy, et al 2018.[16]

Table: 1Percentage of Decolorization of Azo dye

Seed treated with metabolic product of Congo red and Methylene blue		
Degradative Metabolic Product	Relative Germinated Rate (RGR)	Relative Length Rate (RLR)
Congo red by Aspergillus	20%	4.05%
Methylene blue by Aspergillus	80%	5.43%
Congo red by Acinetobacter	60%	5.42%
Methylene Blue by Acinetobacter	40%	2.43%
Congo red by Aspergillus & Acinetobacter	40%	24.40%
Methylene blue by Asper & Acineto	60%	3.35%

Table:2 Seed germination assay:

Percentage of Decolorization of Azo dye (0.5%)			
Dye	By *Aspergillus spp.*	By *Acinetobacter spp.*	Mix *Aspergillus spp.* and *Acinetobacterspp.*
Congo red	59.53%	42.3%	61.53 %
Methylene blue	38.00%	45.41%	47.7%

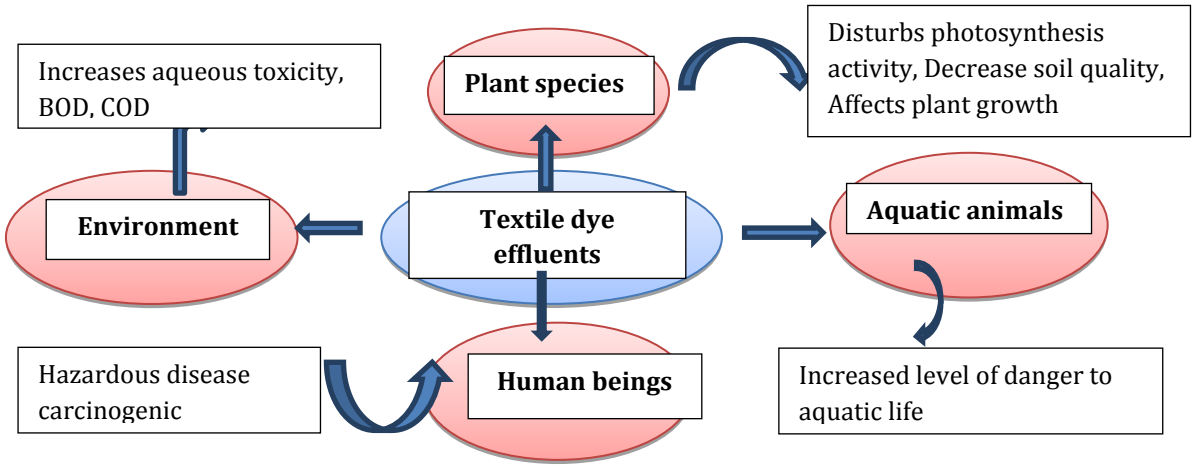


Fig. 1.1 Effects of textile effluent [14]

Fig. 1.3 Application of Biological treatment of dye containing wastewater [15]

Fig. 3.1.1De colorization Zone around Fungi

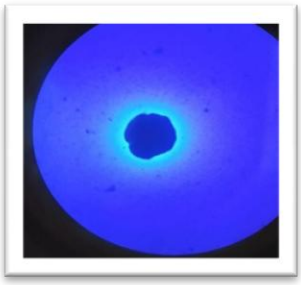


Fig. 3.1.2 De colorization Zone around bacteria

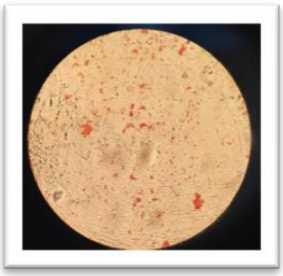


Fig. 3.1.3 Gram Staining of Bacterial Strain

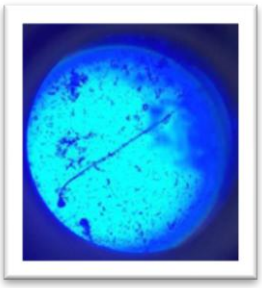
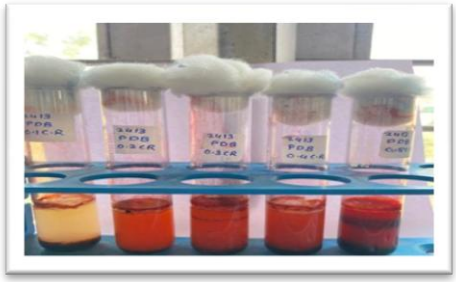
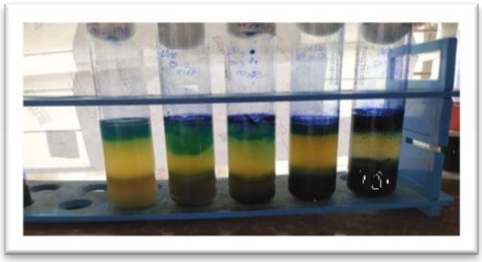


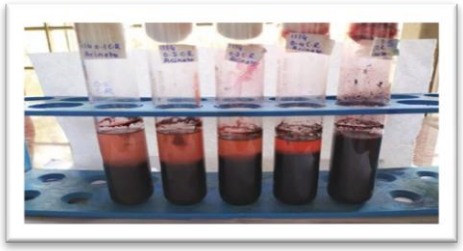
Fig. 3.1.4Cotton Blue Staining of Fungal strain



3.2.1De colorization in Congo red using *Aspergillus spp.*



3.2.2Decolorization in Methylene blue using *Aspergillus spp.*



3.2.3De colorization in Congo red using *Acinetobacter Spp.*

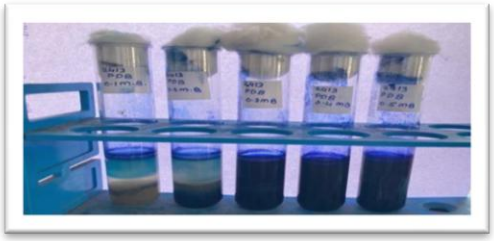


Fig.3.2.4 Decolorization in Methylene blue using *Aspergillus spp.*

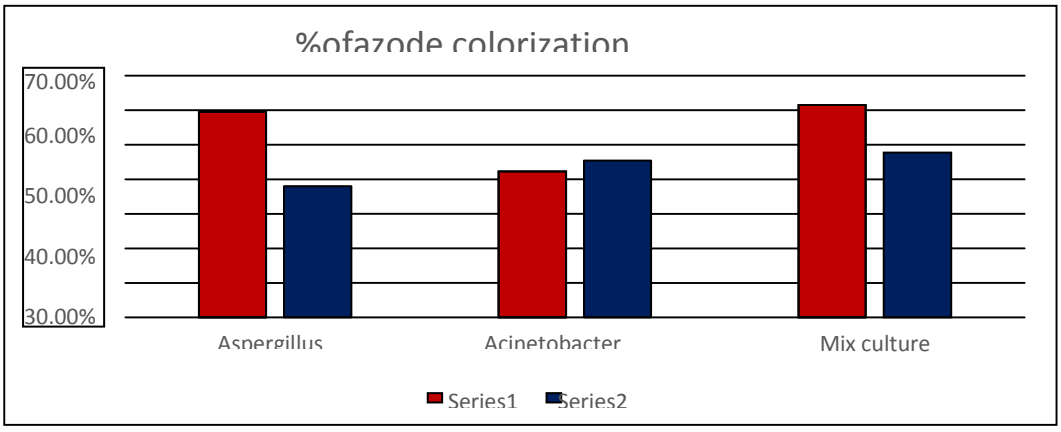
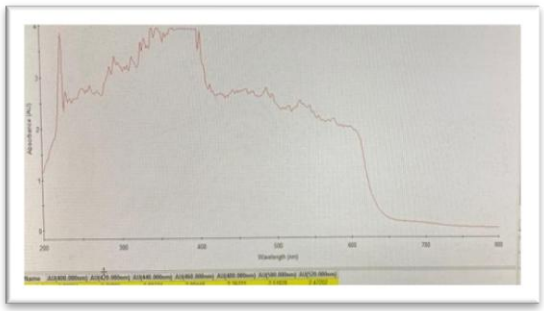


Fig. 3.3.1Statistical analysis of dye de colorization.



3.4.1UV Spectra of Congo red

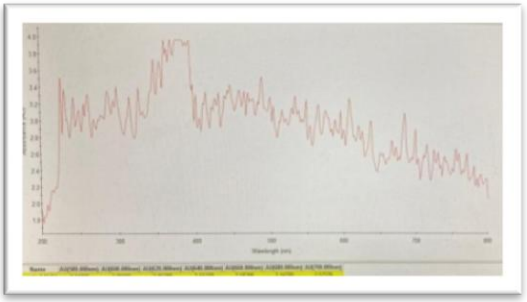


Fig. 3.4.2 UV Spectra of Methylene blue

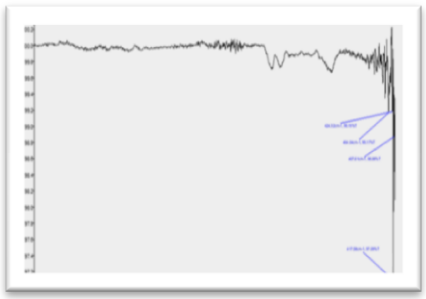


Fig. 3.5.1 FTIR Analysis *Aspergillus spp*. before Treatment

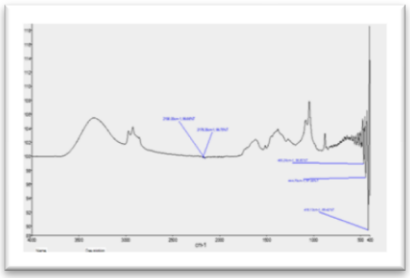


Fig. 3.5.2 After Treatment *Aspergillus spp.* in Congo red

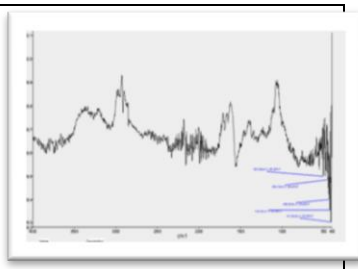


Fig. 3.5.3 After Treatment *Aspergillus spp.* in Methylene blue

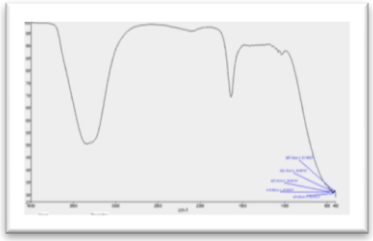


Fig. 3.5.4 FTIR Analysis of *Acinetobacterspp.*before Treatment

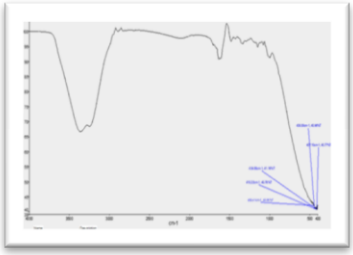


Fig. 3.5.5 After Treatment *Acinetobacterspp.*in Congo red

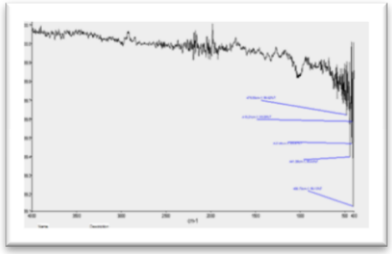


Fig. 3.5.6 After Treatment by Methylene Blue By *Acinetobacter spp.*

3.4 Phytotoxicity assay:

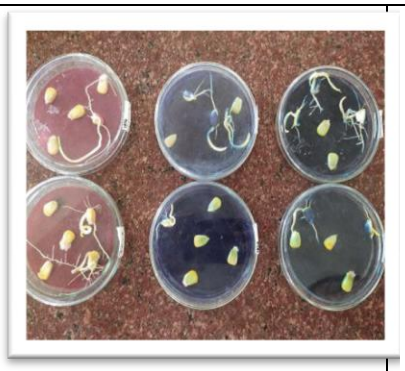


Fig.3.4.1 Phytotoxicity Assay using *Zeamaize* growth after 10 days before decolorization of azo dye

Fig. 3.4.2 Phytotoxicity Assay using *Zeamaize*growth after 10 days using distilled water (Positive control)

Fig. 3.4.3 Phytotoxicity Assay using *Zeamaize*growth after 10 daysafter decolorization of azo dye

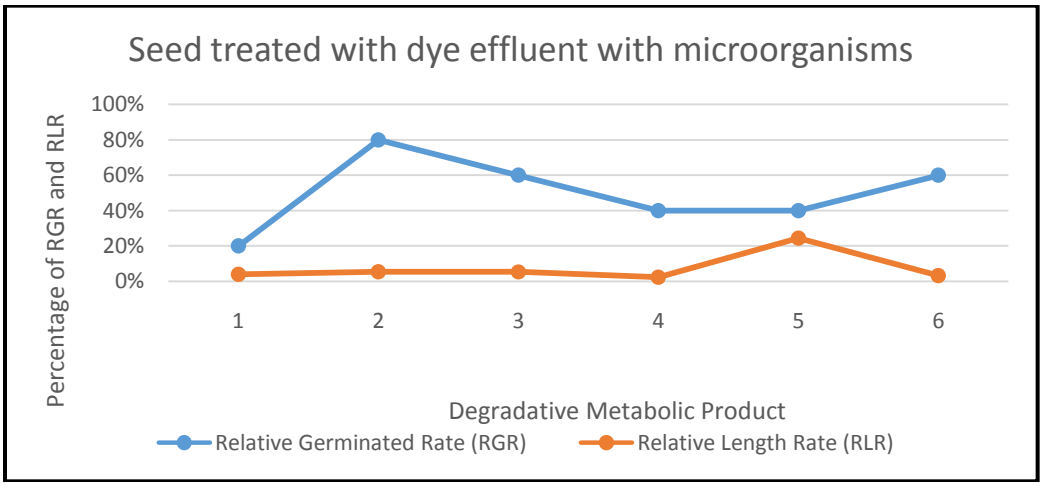


Fig. 3.4.4 Statistical analysis of phytotoxicity assay

DECLARATIONS

Acknowledgements:
I acknowledged principal, P.D.E.A's Annasaheb Magar College, Hadapsar, HOD of Microbiology Department Prof. N.N. Patil, Guide Dr. S.R. Shinde, all staff members of Microbiology department, Annasaheb Magar College, and family members.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author's Contribution: Main laboratory work has been done by Dhangar U.S, Ghule A.V[1], Moundekar A. A. Research article is contributed by Dhangar U.S and Guide Dr. Shinde S.R.

Funding: Self-funding.

Ethics Statement: No involvement of humans and animals for any experiment.

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CITATION OF THIS ARTICLE

U. S. Dhangar, S. R Shinde, N. N. Patil, A.V. Ghule and A. A. Moundekar: Bioremediation of Azo dyes by microbial consortia isolated from textile effluent contaminated soil, Bull. Env. Pharmacol. Life Sci., Spl Issue [1]: 2023:317-324.