The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(3): S.I(3). 1631-1651, 2025 www.thebioscan.com

The Application and Assessment of Textile Dye Golden Yellow HER by Bacteria and its Impact on Seed Germination

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DOI: 10.63001/tbs.2025.v20.i03.S.I(3).pp1631-1651

KEYWORDS:
Biodegradation, Golden Yellow HER, MANOVA, two-way ANOVA, enzyme activity, phytotoxicity

Received on:
21-07-2025

Accepted on:
30-09-2025

Published on:
12-11-2025

ABSTRACT

Two-way Multivariate Analysis of Variance (MANOVA) is a statistical method used to assess how two independent variables jointly influence two or more continuous dependent variables. It allows for the evaluation of both main and interaction effects. In the present study, two-way MANOVA was applied to investigate the biodegradation of a textile dye. The textile industry uses a substantial amount of dyestuffs for fabric coloration, and a large portion of these dyes is discharged through effluents, posing serious environmental hazards. Conventional effluent treatment methods are often ineffective in completely removing such dyes, making biological degradation a preferred alternative.

In this research, the commonly used textile dye Golden Yellow HER was selected, and five newly isolated bacterial strains (GYH-1 to GYH-5) were evaluated for their biodegradation potential. These strains were obtained from acclimatized samples and identified using morphological, biochemical, and 16S rRNA sequencing techniques. Quantitative enzymatic activity assays revealed that both bacterial strain and incubation time had significant effects on enzyme activity, with a significant interaction between the two factors as well. The enzymes considered for present study are Azoreductase, Manganese Peroxidase, and Laccase Furthermore, phytotoxicity studies were conducted on *Jowar* and *Mung* seeds, where two parameters two parameters radicle length and plumule length are considered. It is also statistically tested whether the treated dye water, after degradation, is as safe for plants as distilled water.

INTRODUCTION

Two-way Multivariate Analysis of Variance (MANOVA) is an extension of the MANOVA technique that allows the simultaneous study of the influence of more than one independent variables on two dependent variables. Unlike one-way MANOVA, which considers only a single factor, two-way MANOVA examines not only the main effects of both factors but also their possible interaction effects on the set of

response variables. This technique is widely applied in fields like textiles, medicine, agriculture, psychology, and microbiology; where treatments are often influenced by more than one factor and multiple characters need to be analyzed together to obtain important results.

Schott (2007) contributed to the field by developing high-dimensional tests for one-way MANOVA [1]. Now a days pollution from the chemicals and heavy metals has grown into a worldwide difficulty because they are toxic and everlasting to living being in the environment [2]. Aromatic sulfo and azo groups are man-made chemical structures; therefore, azo dyes are classified as xenobiotic compounds. Due to their complex and stable molecular structure, these dyes are highly resistant to degradation and are commonly found in large quantities in textile industry wastewater [3]. Textile dyes are highly resistant by nature and cannot be easily broken down using common treatment methods. Although extensive research has been carried out to remove these dyes with promising results, most studies have focused only on a single model compound [4, 5]. Real textile wastewater contains a complex mixture of various dyes in unknown proportions [6]. In the environmental sciences the use of microbiological agents or bioremediation techniques to cope up with pollution problem is a strategic research area. The tool for the biodegradation of recalcitrant dye in the textile effluent is based on biotransformation by microbial enzymes [7, 8]. At present, microbial or enzymatic treatments are commonly used for the bioremediation of textile dyes because they offer several advantages. These methods are cost-effective, eco-friendly, and require less water compared to physical treatment processes. Moreover, the byproducts formed after treatment are either non-toxic or fully mineralized, and the process generates very little sludge [9]. The improvement of compelling cultures and their use in degradation is one of the best biological features of effluent treatment [10]. Bioremediation is a natural process that uses the abilities of beneficial microbes, often enhanced through bioengineering, to clean up the environment. It serves as an effective and eco-friendly alternative to traditional cleanup methods [11]. In our natural environment, bacterial communities found in different micro-niches are remarkable life forms capable of using a wide variety of compounds as their sources of carbon and energy [12]. Textile effluents containing azo dyes are not only resistant to degradation but

also exhibit phytotoxic, toxic, carcinogenic, and mutagenic effects [13, 14]. Although significant time and resources have been invested, making bioremediation widely accessible still remains a major challenge today.

The present research focuses on utilizing a diverse range of bacterial cultures capable of breaking down the complex structure of the textile dye Golden Yellow HER and simultaneously detoxifying it, while sustaining continuous growth with minimal nutrient requirements. In this article the application of two way MANOVA in textile industries for biodegradation of textile dye is explored and its impact on germination of Jowar and Mung seeds is studied.

MATERIALS AND METHODS

The impact of five bacterial strains GYH-1, GYH-2, GYH-3, GYH-4, and GYH-5 on the Golden Yellow HER dye was evaluated at two time intervals such as 24 hours and 48 hours. The textile dye Golden Yellow HER was procured from Sigma-Aldrich (USA). The experiment was designed statistically. In the present study the degradation efficiency of textile dye Golden Yellow HER by five potent bacterial isolates *viz*. GYH-1, GYH-2, GYH-3, GYH-4 and GYH-5 were considered. The textile dye Golden Yellow HER was treated by using these bacteria in simple nutrient medium. Activity of various Oxidoreductase enzymes for dye degradation was also determined. Manganese Peroxidase and Azoreductase enzymes were responsible for cleavage of dye structure. Mainly Azoreductase initiates the metabolic cleavage of dye Golden Yellow HER by cleavage of azo bridge [15]. Phytotoxicity studies revealed that the bacterial isolates degraded toxic and harmful dye into non-toxic and safe metabolites.

Sample collection

Soil and water samples were collected from areas near waste discharge sites of various textile industries in Pune, Maharashtra (India). The samples were then stored in sterile plastic containers for further analysis.

Acclimatization of samples

To obtain an active microbial community capable of efficiently removing dyes from textile effluents, the collected soil and water samples were thoroughly mixed, homogenized, and gradually exposed to increasing concentrations of Golden Yellow HER dye over a period of one month. These

acclimated samples were then used to isolate the most effective dye-degrading bacteria.

Isolation and identification of promising bacterial isolates

Nutrient agar medium containing 100 ppm of Golden Yellow HER dye was used for isolation and screening of dye degrading bacteria. Total 15 bacterial isolates were isolated among all 5 isolates were showing highest decolorization and primarily designated as GYH-1, GYH-2, GYH-3, GYH-4 and GYH-5. Identification of selected bacteria was done by routine morphological, biochemical and 16S rRNA sequencing.

Statistical Analysis for determination of enzyme activity

Certain oxidoreductive enzymes, including azoreductase, manganese peroxidase, and laccase, play a key role in the bioremediation of textile dyes[16, 17, 18, 19, 20]. Quantitatively Azoreductase activity was determined by 0.1 mM NADH [21, 22], Manganese Peroxidase enzyme activity assay was performed by using Guaiacol as a substrate [23] whereas activity of Laccase enzyme was determined by using 0.5 mM ABTS [2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] in 1 mM (pH 5.0) Sodium Acetate Buffer. The reaction kinetics was monitored by spectrophotometer at 420 nm (Shimadzu-UV-3600 Plus) at 30^0C for 5 minutes. All the assays were performed in triplicate. One unit of enzyme activity was expressed as 1 µmol of ABTS oxidized per minute ($\varepsilon 420 = 3.6 \times 104\ M^{-1}\ Cm^{-1}$) [24].

RESULTS

An experiment was conducted to evaluate the effect of five bacterial strains (GYH-1, GYH-2, GYH-3, GYH-4, and GYH-5) at two time intervals (24 hrs and 48 hrs) on the degradation of Golden Yellow HER dye. Three enzyme activities—Azoreductase, Manganese Peroxidase, and Laccase—were measured as dependent variables. The independent variables in this study were bacterial strain and incubation time. To assess the multivariate effects of bacteria, time, and their interaction on enzyme activities, a two-way Multivariate Analysis of Variance (MANOVA) was performed using Pillai's trace test in R software. The MANOVA results are presented in Table 1.

Table1: Two-way MANOVA for Enzyme Activity of 5 types of bacteria and time

Source	Df	Pillai	Approx. F	Num Df	Den Df	P value
Bacteria	4	2.2084	13.95	12	60	0.0015
Time	1	0.9903	610.76	3	18	0.0024
Bacteria: Time	4	2.3616	18.5	12	60	0.0078
Residuals	20					

From table 1, It was observed that the p-value for the effect of bacterial activity was 0.0015 (< 0.05)**,** indicating a statistically significant difference among at least two of the enzyme activities—Azoreductase, Manganese Peroxidase, and Laccase. p-value for time is 0.0024 < 0.05 indicating significant difference in enzyme activity with respect to time. Also the interaction effect of various bacteria and time is statistically significant (p-value = 0.0078 < 0.05); hence the bacteria effects and time effects cannot be clearly interpreted. Hence, the two-way ANOVA is performed separately for each enzyme activity. Results are given in Table 2.

Table 2: Two Way ANOVA for Azoreductase, Manganese peroxide and Laccase enzyme activity

2.a Azoreductase Enzyme activity

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	2.6064	0.6516	94.71	0.0007
Time	1	2.5056	2.5056	364.19	< 0.0001
Bacteria : Time	4	1.6931	0.4232	61.52	< 0.0001
Residuals	20	0.1376	0.00688		

2.b Maganese enzyme activity

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	6.8511	1.7128	335.618	< 0.0001
Time	1	2.3185	2.3185	454.315	0.0003
Bacteria : Time	4	1.1819	0.2954	57.899	< 0.0001
Residuals	20	0.1021	0.0051		

2.c Laccase Enzyme activity

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	3.6958	0.9239	136.21	0.0003
Time	1	0.867	0.867	127.813	0.0002
Bacteria : Time	4	1.0354	0.2589	38.161	0.0004
Residuals	20	0.1357	0.0068		

From the above table 2.a,2.b and 2.c; p - value for the bacteria is (< 0.005) for all the three enzyme activities, therefore the effect of various bacteria on Azoreductase, Manganese and Laccase enzyme activities are statistically significant. p - value for the main effect of time and interaction effect of bacteria and time is (< 0.005) for all three enzyme activities therefore the effect of time is statistically significant on this Azoreductase, Manganese and Laccase enzyme activities. Also the interaction effect is significant for all the enzyme activities. It is of interest to identify which specific bacteria at what time show significant differences in association with the enzymes Azoreductase, Manganese Peroxidase and Laccase. Hence multiple comparison tests are conducted using R software. The results are enlisted in Table 3.

Table 3: Multiple Comparisons of action of Azoreductase, Manganese peroxide and Laccase enzymes from 5 bacterial isolates

Azoreductase enzyme			**Manganese peroxide enzyme**			**Laccase enzyme**		
Bacteria	diff	p adj	Bacteria	diff	p adj	Bacteria	diff	p adj

GYH-2 -GYH-1	-0.57	0.0398	GYH-2 -GYH-1	-0.450	0.229	GYH-2 -GYH-1	-0.030	0.9430
GYH-3 -GYH-1	-0.73	0.0107	GYH-3 -GYH-1	-0.66	0.0592	GYH-3 -GYH-1	-0.330	0.0608
GYH-4 -GYH-1	-1.32	0.0002	GYH-4 -GYH-1	-0.790	0.0138	GYH-4 -GYH-1	-0.020	0.9989
GYH-5 - GYH -1	-0.390	0.1523	GYH-5 - GYH -1	0.140	0.8215	GYH-5 - GYH -1	0.3300	0.0234
GYH-3 -GYH -2	-0.160	0.8866	GYH-3 -GYH -2	-0.21	0.8005	GYH-3 -GYH -2	-0.300	0.0105
GYH-4 - GYH-2	-0.750	0.0052	GYH-4 - GYH-2	-0.34	0.6692	GYH-4 - GYH-2	0.010	0.9998
GYH-5 - GYH-2	0.180	0.7809	GYH-5 - GYH-2	0.590	0.0802	GYH-5 - GYH-2	0.36	0.031
GYH-4 - GYH-3	-0.590	0.0454	GYH-4 - GYH-3	-0.13	0.9305	GYH-4 - GYH-3	0.330	0.0534
GYH-5 - GYH-3	-0.010	0.9961	GYH-5 - GYH-3	0.80	0.0309	GYH-5 - GYH-3	0.690	0.06
GYH-5 - GYH-4	0.580	0.0304	GYH-5 - GYH-4	0.930	0.0092	GYH-5 - GYH-4	0.360	0.0003

From Table 3, GYH-1 bacteria show a significant difference ($p < 0.05$) in Azoreductase enzyme activity compared to GYH-2, GYH-3, and GYH-4. Additionally, GYH-4 and GYH-5 also differ significantly in Azoreductase activity.

For Manganese peroxidase activity, significant differences are observed ($p< 0.05$) between GYH-4 and GYH-1, as well as between GYH-5 and GYH-3, and GYH-5 & GYH-4.

In terms of Laccase enzyme activity, GYH-5 shows a significant difference compared to all other bacteria.

Table 4: Multiple Comparisons of action of Azoreductase, Manganese peroxide and Laccase enzymes from two time periods

Time: A: 24 Hrs B: 48 Hrs

Enzyme Activity	Time	Difference	P value
Azoreductase	B – A	-0.578	0.0001
Manganese peroxide	B – A	-0.556	0.0002
Laccase	B – A	-0.34	0.003

From Table 4, all three enzyme activities show the highest impact at 24 hours. Among them, Azoreductase activity exhibits the greatest difference, followed by manganese peroxidase and laccase.

Phytotoxicity studies of degraded products:

The phytotoxicity study was performed by observing seed germination with two types of seeds. Jowar (*Sorghum vulgare*) and Mung (*Vignaradiata*) are used for phytotoxicity

study of treated dye products. These seeds were added with 1ml of original dye solution and 1ml treated dye solution separately in respective Petri plates. In the third set the seeds were added with 1ml distilled water which was used as control for this test. These plates were kept at room temperature. All the samples were added for consecutive 7 days. The length of root and shoot was recorded and compared with control distilled water.

The sampled water is the broth containing degraded dye metabolites by the bacterial enzymes (treated dye sample) and original dye sample. Here we have taken distilled water as a control group and checked the effect of treated dye sample from five bacteria GYH-1, GYH-2, GYH-3, GYH-4 and GYH-5 on the plant of Jowar (*Sorghum vulgare*) and Mung beans (*Vignaradiata*). The parameters considered for present study are length of radicle and length of plumule. The length of plumule and radical were measured in millimeters manually by ruler [25]. Two-way MANOVA is conducted for testing the effect of bacteria and sampled water on the plant parameters like radicle and plumule. Here the two independent variables are bacteria and sampled water. Two dependent variables are length of radicle and length of plumule. The output for present study is given in Table 5.

Table 5: Two Way MANOVA for the plant parameter of Jowar

Source	Df	Pillai	Approx. F	Num Df	Den Df	P value
Bacteria	4	1.0791	8.789	8	60	< 0.0001
Sampled water	2	1.2954	27.58	4	60	< 0.0001
Bacteria: Sampled water	8	1.3720	8.1924	16	60	< 0.0001
Residuals	30					

From Table 5, The p-value for the bacterial effect is less than 0.0001, indicating a statistically significant impact on the plant parameters of Jowar viz. length of radicle and length of plumule. Similarly, the p-value for the sampled water is also below 0.0001, demonstrating a significant effect on the plant parameters. Also the interaction effect of different bacteria and sampled water (p-value < 0.0001) is statistically significant hence the bacteria effects and sampled water effect cannot be clearly interpreted**.** Hence,

two-way ANOVA is conducted separately for each plant parameter. Output of two-way ANOVA is enlisted in Table 6.

Table 6: Two Way ANOVA for Plant parameter of Jowar

Here bacterial strains like GYH-1, GYH-2, GYH-3, GYH-4 and GYH-5 and sampled water like distilled water, treated dye water and original dye are independent variables, whereas length of radicle and length of plumule for the plant of Jowar are dependent variables.

6.a Two Way ANOVA for Length of Radicle as dependent variable

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	1.5513	0.3878	9.1271	< 0.0001
Sampled water	2	0.8338	0.4169	9.8094	< 0.0001
Bacteria: Sampled water	8	2.089	0.2611	6.1453	0.0042
Residuals	30	1.2747	0.0425		

6.b Two Way ANOVA for Length of Plumule as dependent variable

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	3.93	0.982	58.7633	< 0.0001
Sampled water	2	72.341	36.171	227.4906	< 0.0001
Bacteria: Sampled water	8	1.275	0.159	9.6303	0.0021
Residuals	30	0.497	0.017		

From Table 6.a and 6.b, effect of bacteria, sampled water and also the interaction effect are significant (p -value < 0.0001) on the plant parameter like length of radicle and length of plumule. Multiple comparison tests are applied for finding exactly which pairs differ significantly. The multiple comparison tests for Distilled water, Treated dye sample

and Original dye with respect to each bacterium on radicle length are conducted in R; output of it is listed in Table 7. Similarly output of multiple comparison test for the plumule length is listed in Table 8

Table 7: Multiple comparison test results for interaction between bacteria and sampled water on radical length of Jowar plant

Bacteria	Contrast	Diff.	p-value	Significance	Interpretation
GYH-1	Distilled – Original	1.340	<.0001	Significant	Distilled > Original
	Distilled – Treated	-0.050	0.9526	Not significant	Distilled ≈ Treated
	Original – Treated	-1.390	<.0001	Significant	Treated > Original
GYH-2	Distilled – Original	1.516	<.0001	Significant	Distilled > Original
	Distilled – Treated	0.183	0.528	Not significant	Distilled ≈ Treated
	Original – Treated	-1.330	<.0001	Significant	Treated > Original
GYH-3	Distilled – Original	0.837	<.0001	Significant	Distilled > Original
	Distilled – Treated	0.337	0.1295	Not significant	Distilled ≈ Treated
	Original – Treated	-0.500	0.0155	Significant	Treated > Original
GYH-4	Distilled – Original	0.896	<.0001	Significant	Distilled > Original
	Distilled – Treated	0.286	0.2206	Not significant	Distilled ≈ Treated
	Original – Treated	-0.610	0.003	Significant	Treated > Original
GYH-5	Distilled – Original	0.637	0.0019	Significant	Distilled > Original
	Distilled – Treated	0.593	0.0038	significant	Distilled > Treated
	Original – Treated	-0.043	0.9642	Not Significant	Treated ≈ Original

From Table 7, p-value for the comparison of distilled water and original dye water for all bacteria (< 0.0001) showing the significant effect of distilled water and original dye water on the length of radicle of Jowar plant. The difference show that the length of radicle in distilled water treatment consistently yielded significantly higher bacterial responses than the original dye water, with differences ranging from 0.637 to 1.516. Differences between distilled water and treated dye water treatments were small and statistically insignificant, indicating there is no significant difference between distilled water and treated dye water (p-value > 0.05),

indicating both are equally effective on the radicle length of Jowar plant.

Table 8: Multiple comparison test results for interaction between bacteria and sampled water on plumule length of Jowar plant

Bacteria	Contrast	Diff.	p-value	Significance	Interpretation
GYH-1	Distilled - Original	2.123	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	0.08	0.7291	Not significant	Distilled ≈ Treated
	Original - Treated	-2.043	< 0.0001	Significant	Treated > Original
GYH-2	Distilled - Original	2.98	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	-0.183	0.2055	Not significant	Distilled ≈ Treated
	Original - Treated	-3.163	< 0.0001	Significant	Treated > Original
GYH-3	Distilled - Original	2.803	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	0.113	0.5343	Not significant	Distilled ≈ Treated
	Original - Treated	-2.69	< 0.0001	Significant	Treated > Original
GYH-4	Distilled - Original	2.99	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	0.14	0.3887	Not significant	Distilled ≈ Treated
	Original - Treated	-2.85	< 0.0001	Significant	Treated > Original
GYH-5	Distilled - Original	2.717	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	0.187	0.1945	Not significant	Distilled ≈ Treated
	Original - Treated	-2.53	< 0.0001	Significant	Treated > Original

From Table 8, p-value for the comparison of distilled water and original dye water for all bacteria is < 0.0001; hence the effect of distilled water and original dye water on the plumule length of Jowar plant is highly significant. The difference show that the length of plumule in distilled water treatment consistently yielded significantly higher bacterial responses than the original, with differences ranging from 2.123 to 2.990. Differences between distilled water and treated dye water treatments were small and statistically insignificant, indicating there is no significant difference between distilled water and treated dye water (p-value > 0.05), indicating both are equally effective on the plumule length of Jowar plant.

Figure 1: Phytotoxicity studies of treated and untreated Golden Yellow HER dye on Jowar (*Sorghum vulgare*)

Table 9: Two way MANOVA for the plant parameter of Mung beans

Source	Df	Pillai	Approx. F	Num Df	Den Df	P value
Bacteria	4	1.3005	13.945	8	60	0.0045
Sampled water	2	1.432	37.828	4	60	0.0008
Bacteria: Sampled water	8	1.1772	5.365	8	60	0.0077
Residuals	30					

From Table 9, p-value for bacteria = 0.0045 < 0.05; hence effect of bacteria is significant on the plant parameter of Mung beans like length of radicle and length of plumule. The

effect of sampled water is statistically significant on the parameters of Mung beans like radicle and plumule (p -value = 0.0008 < 0.05). The interaction effect of different bacteria and sampled water (p-value = 0.0077 < 0.05) is also statistically significant. Hence the bacteria effects and sampled water effect cannot be clearly interpreted**.** Therefore, the Two-way analysis of variance is applied for each plant parameter of mung beans in table 10.

Table 10: Two way ANOVA for plant parameter of Mung beans

Here bacterial strains like GYH-1, GYH-2, GYH-3, GYH-4 and GYH-5 and sampled water like distilled water, treated dye water and original dye are independent variables, whereas length of radicle and length of plumule for the plant of Mung beans are dependent variables.

10.a Two Way ANOVA for Length of Radicle as dependent variable

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	0.29	0.062	4.6816	0.0046
Sampled water	2	65.086	32.543	2443.17	< 0.0001
Bacteria: Sampled water	8	0.498	0.062	4.673	0.0008
Residuals	30	0.4	0.013		

10.b Two Way ANOVA for Length of Plumule as dependent variable

Source	Df	Sum Sq	Mean Sq	F value	P value
Bacteria	4	3.3	0.824	75.1322	< 0.0001
Sampled water	2	474.59	237.295	2154.801	< 0.0001
Bacteria: Sampled water	8	0.87	0.109	9.956	< 0.0001
Residuals	30	0.33	0.011		

From Table 10.a and 10.b, the p-value for the effect of bacteria on the length of the radicle in mung beans is 0.0046, which is less than the significance level of 0.05. This indicates

that bacteria have a statistically significant effect on radicle length. Similarly, the p-value for the effect of sampled water is less than 0.0001, demonstrating a highly significant impact on radicle length. Moreover, the interaction between bacteria and sampled water also shows a significant effect on radicle length. To identify which specific combinations of bacteria and sampled water have the most pronounced effects on the length of radicle of Mung beans, a multiple comparison test was conducted. The detailed results of this test are presented in Table 11.

The p-value for the effect of bacteria on the length of the plumule in mung beans is < 0.0001, This indicates that bacteria have a highly statistically significant effect on plumule length. Similarly, the p-value for the effect of sampled water is less than 0.0001, demonstrating a highly significant impact on plumule length. Moreover, the interaction effect of bacteria and sampled water also shows a significant effect on plumule length. To identify which specific combinations of bacteria and sampled water have the most pronounced effects on the length of plumule of Mung beans, a multiple comparison test was conducted. The detailed results of this test are presented in Table 12.

Table 11: Multiple comparison test results for interaction between bacteria and sampled water on radicle length of Mung beans.

Bacteria	Contrast	Diff.	p-value	Significance	Interpretation
GYH-1	Distilled - Original	2.913	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	0.00012	0.9918	Not significant	Distilled ≈ Treated
	Original - Treated	-2.913	<0.0001	Significant	Treated > Original
GYH-2	Distilled - Original	2.65	<0.0001	Significant	Distilled > Original
	Distilled - Treated	-0.05	0.857	Not significant	Distilled ≈ Treated
	Original - Treated	-2.7	<0.0001	Significant	Treated > Original
GYH-3	Distilled - Original	2.47	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	0.127	0.3825	Not significant	Distilled ≈ Treated
	Original - Treated	-2.343	0.0155	Significant	Treated > Original
GYH-4	Distilled - Original	2.34	< 0.0001	Significant	Distilled > Original
	Distilled - Treated	-0.02	0.9755	Not significant	Distilled ≈ Treated
	Original - Treated	-2.31	0.00012	Significant	Treated > Original

GYH-5	Distilled - Original	2.470	0.0013	Significant	Distilled > Original
	Distilled - Treated	0.127	0.0385	significant	Distilled ≈ Treated
	Original - Treated	-2.347	<0.0001	Not Significant	Treated > Original

From the Table11, p-value for the comparison of distilled water and original dye water for all bacteria (< 0.0001) hence the effect of distilled water and original dye water on the length of radicle of Mung beans plant is highly significant. The difference show that the length of radicle in distilled water treatment consistently yielded significantly higher bacterial responses than the original dye, with differences ranging from 2.347 to 2.913. Differences between Distilled water and Treated dye water treatments were small and statistically insignificant, indicating there is no significant difference between Distilled water and Treated water, indicating both are equally effective on the length of radicle of Mung beans plant.

Table 12: Multiple comparison test results for interaction between bacteria and sampled water on plumule length of Mung beans

Bacteria	Contrast	Estimate	p-value	Significance
GYH-1	Distilled - Original	7.473	< 0.0001	Significant
	Distilled - Treated	0.43	0.0001	Significant
	Original - Treated	-7.043	< 0.0001	Significant
GYH-2	Distilled - Original	7.06	< 0.0001	Significant
	Distilled - Treated	0.463	< 0.0001	Significant
	Original - Treated	-6.597	< 0.0001	Significant
GYH-3	Distilled - Original	7.083	< 0.0001	Significant
	Distilled - Treated	0.727	< 0.0001	Significant
	Original - Treated	-6.357	< 0.0001	Significant
GYH-4	Distilled - Original	6.9	< 0.0001	Significant
	Distilled - Treated	0.82	< 0.0001	Significant
	Original - Treated	-6.08	< 0.0001	Significant
GYH-5	Distilled - Original	7.333	< 0.0001	Significant
	Distilled - Treated	0.567	< 0.0001	Significant
	Original - Treated	-6.767	< 0.0001	Significant

From the Table 12, p-value for the comparison of distilled water and original dye water for all bacteria (< 0.0001) hence the effect of distilled

water and original dye water on the plumule length of Mung beans plant is highly significant across all bacterial strains GYH1 to GYH-5. Distilled water consistently leads to significantly greater plumule lengths compared to original dye water, with large positive differences (ranging from 6.9 to 7.47). P-value for the comparison of distilled water and treated dye water for all bacteria (< 0.0001) hence the effect of distilled water and treated dye water on the plumule length of Mung beans plant is highly significant across all bacterial strains (GYH-1 to GYH-5), although the differences are smaller than the original dye (estimates between 0.43 and 0.82). For the comparison of treated dye water and original dye for all bacteria (P-value < 0.0001) hence the effect of treated dye water and original dye on the plumule length of Mung beans plant is highly significant across all bacterial strains (GYH-1 to GYH-5), treated dye water produces significantly longer plumules than original water (negative differences), indicating improvement through treated dye water.

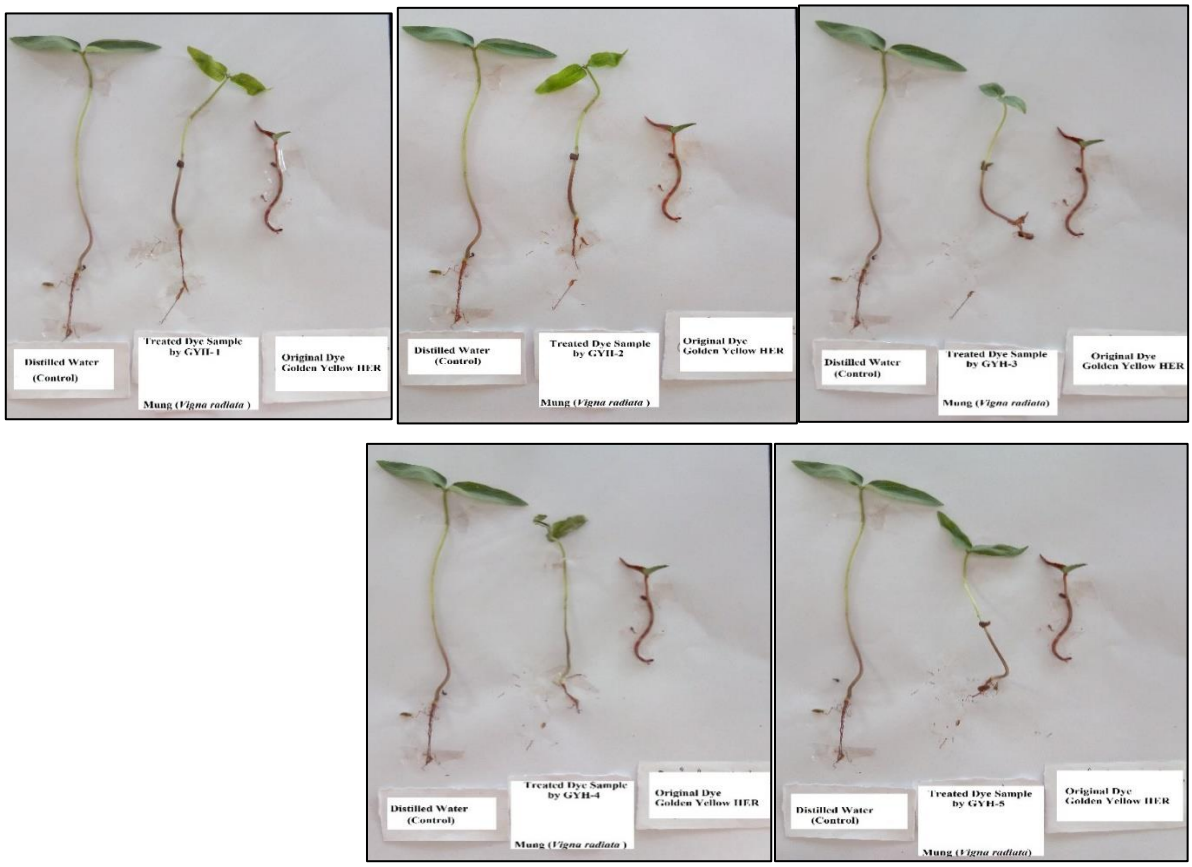

Figure 2: Phytotoxicity studies of treated and untreated Golden Yellow HER dye on Mung beans (*Vignaradiata*)

DISCUSSION

Enzyme activity is statistically significant on the bacteria and time. Also the interaction effect of bacteria and time is significant. Study on Azoreductase enzyme activitiy shows that, GYH-1 differs significantly from GYH-2, GYH-3, and GYH-4; GYH-4 also differs from GYH-5. For the Manganese Peroxidase enzyme activity, Significant differences are found between GYH-4 & GYH-1, GYH-5 & GYH-3, and GYH-5 & GYH-4. For the Laccase enzyme activity, GYH-5 significantly differs from all other strains. These results highlight the importance of bacterial selection in enhancing enzyme activities relevant to bioremediation. It is also found that bacterial strains significantly influence enzyme activities while time does not. However, the significant interaction between bacteria and time suggests their effects are interdependent.

Phytotoxicity studies confirmed that the degradation products were non-toxic, as evidenced by improved radicle growth in treated samples compared to those exposed to untreated dye. Statistical analysis revealed significant effects of bacterial treatment and sampling time on plant growth parameters, indicating effective detoxification. Overall, these findings suggest that the GYH isolates can serve as potent, eco-friendly agents for the treatment of azo dye–contaminated wastewater, offering both rapid decolorization and safe by-product formation.

CONCLUSION

From the biodegradation study, for the Azoreductase enzyme activity GYH-1 bacteria is highly significant and shows higher impact at 24 hours. For Manganese peroxide and Laccase enzyme activity GYH-5 bacteria shows the higher impact at 24 hours. From the Phytotoxicity study, the results indicate that there is no statistically significant difference in the impact of distilled water and treated dye sample water (after degradation) on the plant parameters like length of plumule and length of radicle. This suggests that the treated dye water, after degradation, is as safe for plants as distilled water under the tested conditions.

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