International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 17-06-2025, Accepted: 17-07-2025, Published: 01-08-2025
Volume 7, Issue 3, 2025, Page No. 22-29

Microbial detoxification of azo dye-laden effluents: Actinomycetes for eco-friendly water management

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Abstract

Toxic effluents containing azo dyes are discharged from various industries, and they adversely affect water resources, soil fertility, aquatic organisms, and ecosystem integrity. They pose toxicity (lethal effect, genotoxicity, mutagenicity, and carcinogenicity) to plants, aquatic organisms, and animals. Considering the potential applications of bioremediation processes in wastewater treatment, the present investigation targets the complete detoxification of Trypan Blue by phytotoxicity studies. Phytotoxicity studies indicated that the extracted metabolites (degraded dye) contain nontoxic metabolites, resulting in good germination rate as well as significant root and shoot length of *Gossypium* spp., *Vigna radiata*, *Cicer arietinum*, *Zea mays*, and *Triticum aestivum*when compared to dye samples (untreated), where inhibition in all these parameters was observed.

Keywords: Azo-dyes, phytotoxicity, bioremediation, Soil fertility, dye metabolites, wastewater treatment

Introduction

The growing demand for textiles has led to a considerable rise in the production and use of synthetic dyes, particularly azo dyes. Azo dyes account for nearly 60-70% of all industrial dyes (Pandey *et al*., 2007) [12]. Azo dyes, categorized by one or more azo (-N=N-) bonds, are widely used due to their chemical stability, resistance to degradation, and vivid coloration (Forgacs *et al*., 2004) [7]. However, their extensive application has raised significant environmental concerns. Textile dye effluents, if untreated, contribute to severe water pollution. This negatively impacts aquatic ecosystems and human health (Zollinger, 2003) [19]. Azo dyes and their degradation byproducts, including aromatic amines, are often cancer-causing, mutagenic, and toxic (Pinheiro *et al*., 2004). Conventional physicochemical methods for dye wastewater treatment, such as coagulation, adsorption, and chemical oxidation, are often expensive, generate secondary pollutants, and are inefficient in completely degrading complex dye structures (Hai *et al*., 2007) [7]. Thus, there is an urgent need for eco-friendly and maintainable bioremediation strategies to manage textile dye wastewater effectively.

Actinomycetes, a diverse group of filamentous, Gram-positive bacteria, have emerged as hopeful candidates for the bioremediation of azo dye-contaminated wastewater. These microorganisms are well-known for their enzymatic potential, producing oxidoreductases such as laccases, azoreductases, and peroxidases. All these play a vital role in azo dye degradation (Cetin &Donmez, 2006) [7]. Actinomycetes, predominantly species from genera such as *Streptomyces*, *Micromonospora,* and *Nocardia*, exhibit significant potential in decolorizing and detoxifying industrial dyes (Charumathi & Das, 2012) [5]. Furthermore, immobilization techniques enhance the efficiency and stability of actinomycete-mediated biodegradation, ensuring sustained enzymatic activity and reusability in wastewater treatment systems (Elisangela *et al*., 2009) [6].

Beyond actual dye degradation, an innovative and sustainable approach is the agricultural reutilization of actinomycete-treated textile wastewater. Once the toxic dye compounds are degraded, the treated effluent often contains residual organic and inorganic nutrients, including phosphorus, nitrogen, and trace elements, which can be beneficial for plant growth (Shah, 2014) [15]. Utilizing this treated effluent for irrigation presents a dual advantage: reducing freshwater consumption and enhancing soil fertility (Kumar *et al*., 2012). However, the viability of such applications depends on rigorous valuations of water quality, toxicity reduction, and plant response to ensure no contrary effects on crops and soil microflora (Ali *et al*., 2009) [1].

This study explores the potential of actinomycete-mediated azo dye degradation and its sustainable application in agriculture. It aims to evaluate the efficiency of actinomycetes in eliminating color and toxicity from textile effluents, evaluate the physicochemical properties of the treated wastewater, and investigate its impact on plant growth and soil health. By integrating bioremediation with agricultural practices, this research contributes to circular economy principles, promoting resource recovery, environmental sustainability, and improved wastewater management in the textile industry.

Materials and methods

Microbial culture conditions

This research work utilizes the actinomycete isolate TB14. It was cultivated on actinomycete isolation agar plates (Agar 15 g/L, L-asparagine 0.1 g/L, dipotassium phosphate 0.5 g/L, ferrous sulphate 0.001 g/L, magnesium sulphate 0.1

g/L, sodium caseinate 2 g/L, sodium propionate 4 g/L, final pH 8.1±0.2). The plates were incubated at 37°C for 5 to 7 days. The pure isolate was inoculated on actinomycetes isolation agar slants and stored at 4°C for further use.

Dye degradation experiment

The dye degradation experiment was carried out using minimal media (0.18% dipotassium hydrogen phosphate, 0.001% iron (II) sulfate heptahydrate, 0.02% magnesium sulphate heptahydrate, 0.4% ammonium chloride, 0.01% sodium chloride) supplemented with trypan blue azo dye in a 10mg/ml concentration. A conical flask (no. 1) containing 100 ml of minimal media with trypan blue was inoculated with TB14 (10 %v/v). Another flask (no. 2) with 100 mL of minimal media supplemented with trypan blue was used as a control. One flask (no. 3) with only 100 mL of minimal media was inoculated with TB14 (10 %v/v). All the flasks wereincubated at 37°Cfor 10 days at 100 rpm on a shaker incubator (Wai, Yusop, and Pahirulzaman, 2020) [18].

At regular intervals to check the dye degradation, 2 mL aliquots were removed from the flasks and centrifuged at 1000 rpm for 10 min. The optical density of the supernatant was measured at 600nm. The percentage of dye degradation was calculated using the following formula:

Degradation rate (%) = (Initial absorbance-final absorbance)/ initial absorbance × 100.

Extraction of dye-degraded metabolites for phytotoxicity analysis

After complete dye removal in flask no. 2, all the flasks were removed from the shaker incubator and subjected to centrifugation. The supernatants from each flask were collected in separate containers. These supernatants were further used for phytotoxicity analysis.

Phytotoxicity analysis with different seeds

The dye removal metabolites were checked for their effect on the germination of different seeds. The seeds under study were cotton, green gram (moong), chickpeas, maize, wheat, etc. The five different seeds were planted in the soil in multiple sets. Each set was treated with various water samples. Seeds of each crop were treated with normal distilled water, tap water, sterilized untreated media containing trypan blue, and dye-degraded media (treated) supernatant separately for 15 days, ensuring proper seed growth and development. The effect of dye-degraded metabolites on growth was checked by analyzing the length of root growth, shoot growth, and germination percentage. (Bilal*et al*., 2016) [3].

The percentage of shoot and root growth compared to the control plant was calculated using the following formula:

Percentage Shoot Length = {(Length of Control Plant/Length of Treated Plant)/ Length of Control Plant} ×100

Result and discussion

Despite the risks to health and the environment, untreated textile effluents are dumped into water bodies and used to irrigate crops(Preethi and Pathy, 2020). The process of microbial dye degradation enables the conversion of harmful pollutants into beneficial substances that can support the growth of vegetation. Hence, the acute toxicity of the treated and untreated dye-containingwastewater has to be checked on the growth of a variety of seeds. The germination percentage for each type of plant and in each type of treatment was 100%. All the plant seeds treated with the dye degraded wastewater showed higher growth in terms of shoot and root growth compared to the intact dye water and normal water.

The metabolites of the microbial growth in the medium and the metabolites produced after the dye degradation activity can be beneficial for the plant growth, resulting in the enhanced growth of the plantlets.

The table no.1 and 2 below display the findings for root growth in cm. and shoot growth in cm. for the replicated experiment. It depicts the average shoot and root lengths for each plant in each treatment type.

Table 1: Average growth of shoot length in different conditions

Type of water used	G herbaceum	V radiata	C arietinum	Zea mays	T aestivum
Tap water	11.33	12.33	11	11.33	7.33
Distilled water	13.33	14	13.66	12	8.33
TB Medium w/o TB14	13.33	11.66	11.33	12.66	9.33
TB Medium with TB14	17	19.66	17	18	15.33

Table 2: Average growth of root length of in different conditions.

Type of water used	Gherbaceum	V radiata	C arietinum	Zea mays	T aestivum
Tap water	3.66	3.33	2.33	4.33	2.66
Distilled water	3	3.33	3.33	4	4.33
TB Medium w/o TB14	7	7.66	3.33	6.33	5
TB Medium with TB14	7.66	7.33	6.66	8.66	6.33

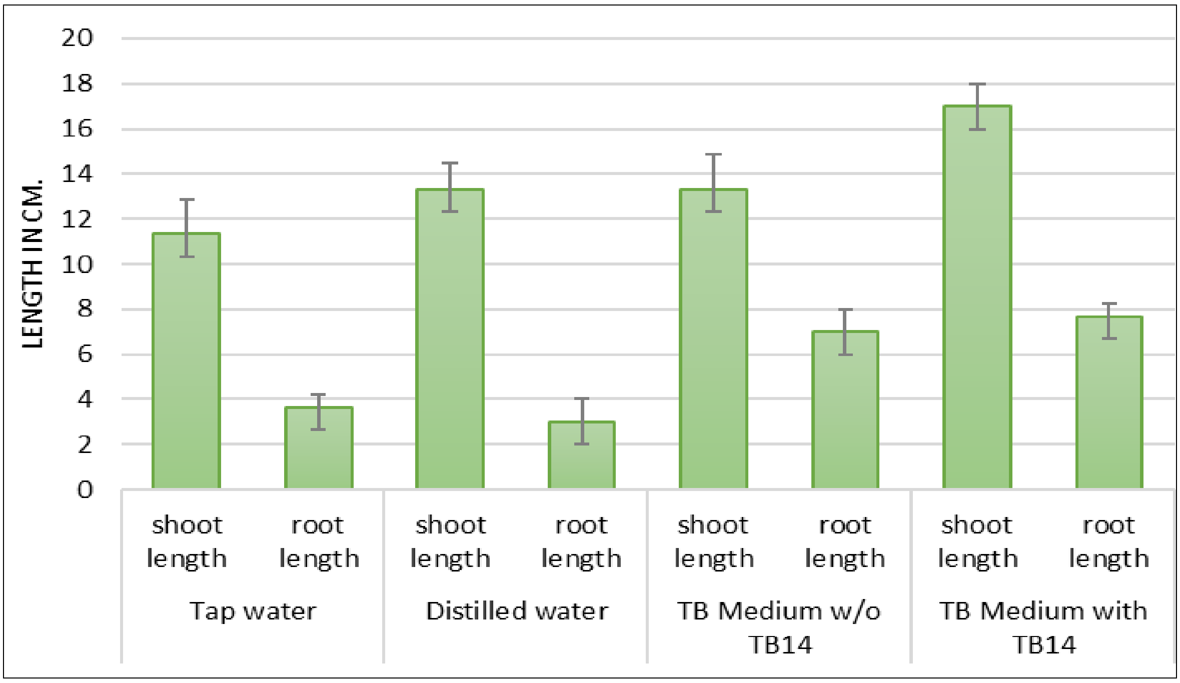
Table 3: Percentage growth of shoot and root length of in different conditions.

Percentage growth		
Plants	**Shoot**	**Root**
G herbaceum	50	109.09
V radiata	59.45	120
C arietinum	54.54	185.71
Zea mays	58.82	100
T aestivum	109.09	137.49

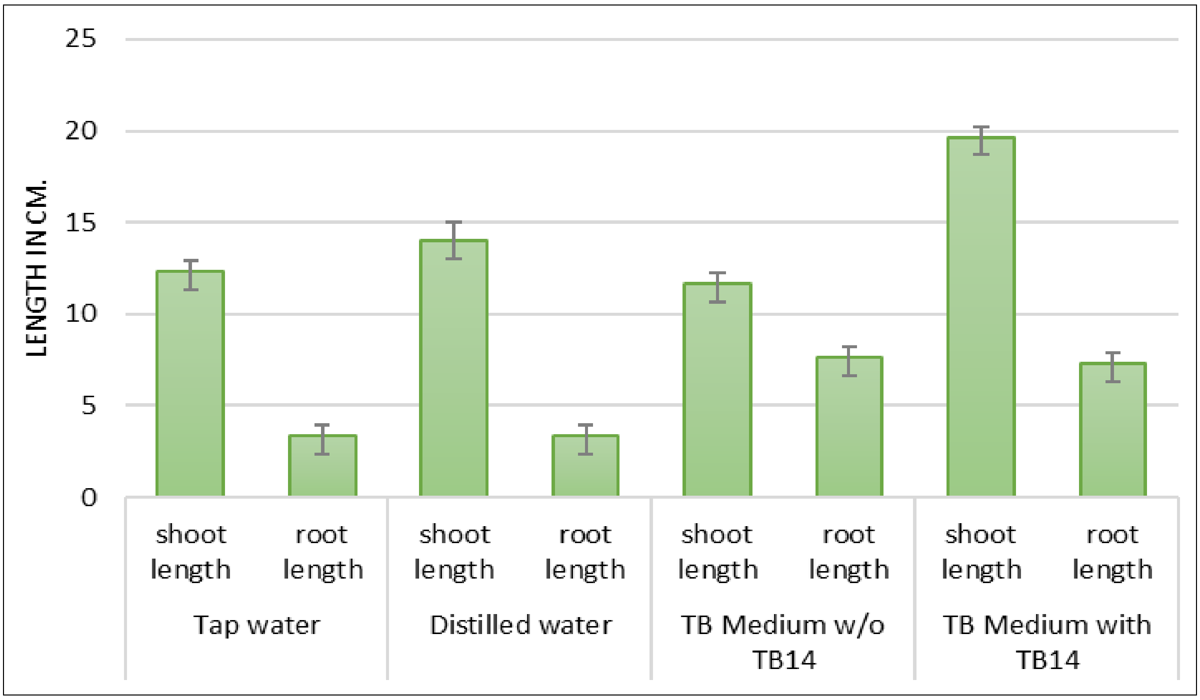
TableNo. 1 shows the specific results for the shoot length for each plant in various conditions. It can be seen that the average growth of shoots among all the plantlets was much higher in *V radiata*. The shoot length for *V. radiata* ranges from 12 cm to 20 cm. The highest growth was found in the plantlet grown in the presence of the dye-degraded medium, i.e., 19.66 cm. The growth of shoots in *Zea mays* when treated with dye-degraded water shows 18 cm. *G. herbacium* and *C arietinum* seeds treated with the degraded dye metabolites showed intermediate shoot length, i.e., 17 cm, whereas the control seeds showed 11.33 cm and 11cm growth, respectively. Much less growth was observed in the plant of *T aestivum* with 15.33cm shoot length.

The seeds treated with the medium containing undegraded dye showed reduced growth in all cases of plants. It may be due to the presence of the toxic nature of the dye, which has an inhibiting effect on plant growth.

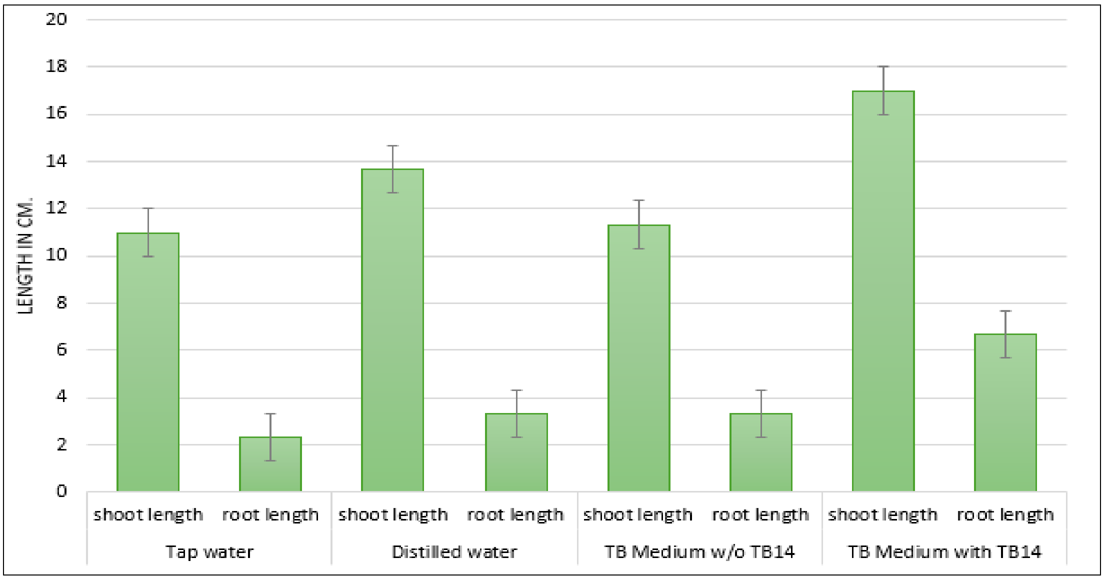
In terms of root growth, TableNo. 2 depicts the results like, *Zea mays* seeds treated with dye degradation metabolites demonstrated a faster growth rate of 8.66 cm. The growth rate was 4.33 cm higher than that of the control seeds. The seeds of *G herbaceum*and *V radiata* exhibited moderate development, measuring 7.66 and 7.33 cm, respectively. *C arietinum* and *T. aestivum* had the smallest root development, measuring 6.66 cm and 6.33 cm, respectively. The metabolites in the dye-degraded sample promoted root development in all kinds of seeds. Reduced growth was seen in the sample with intact dye. This revealed that TB14 biodegraded the dyes, resulting in detoxification and improved plant development in the presence of treated dyes.



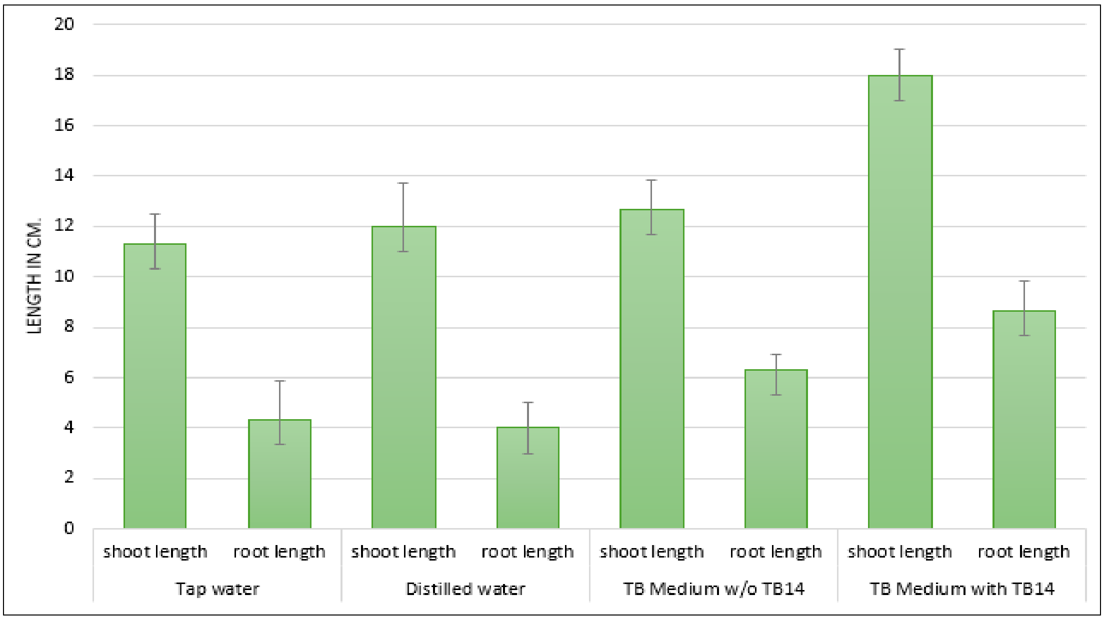
Graph 1: Average shoot and root length of *G herbaceum*seeds under various conditions.



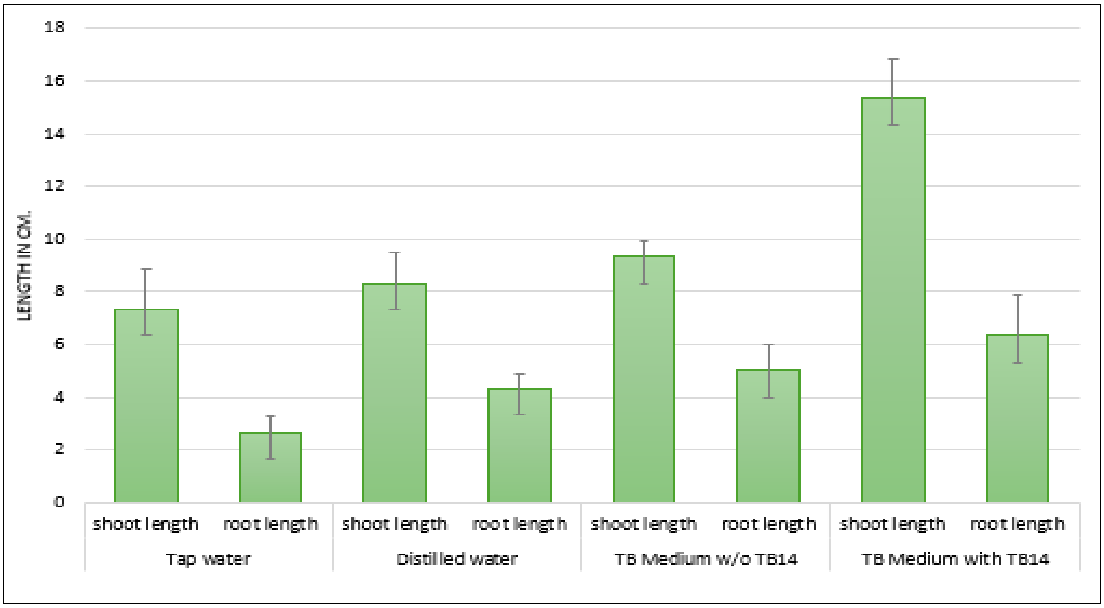
Graph 2: Average shoot and root length of *V radiata* seeds under various conditions.



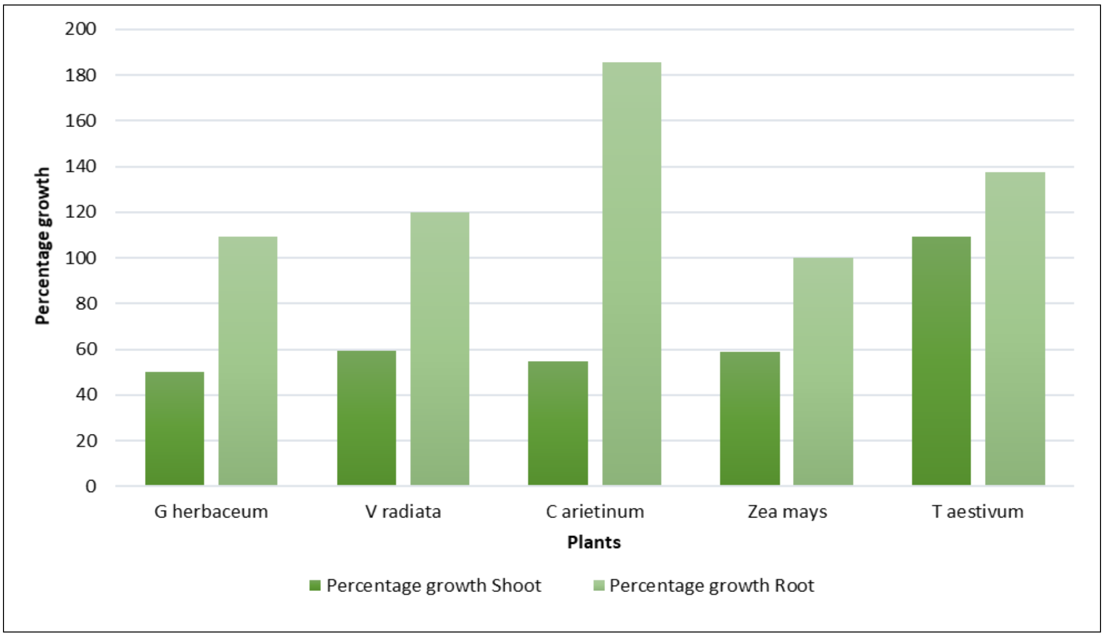
Graph 3: Average shoot and root length of *C arietinum* seeds under various conditions.



Graph 4: Average shoot and root length of *Zea mays*seeds under various conditions.



Graph 5: Average shoot and root length of *T aestivum* seeds under various conditions.



Graph 6: Comparative analysis of growth increase in percentage of root and shoot of each plant.

Table No. 3 presents the comparative analysis of shoot and root growth in different plants treated with different conditions. The highest shoot growth was observed in *Triticum aestivum* among all the plants. This indicates a significant increase in upper-level biomass. The shoot growth observed was 109.09%. A moderate shoot growth was observed in *Cicer arietinum*with 54.54%. A similar result for shoot growth was observed for *Vigna radiata* and *Zea mays*, with 59.45% and 58.82%, respectively. The lowest shoot growth was found in *G. herbaceum*, showing 50% growth only.

The highest root growth was observed in *Cicer arietinum*, showing 185.71% of root growth. A relatively high root growth, i.e., 137.49%, was observed in*Triticum aestivum*. *Vigna radiata*and *Zea mays* display slightly similar root growth percentages, with120% and 100%, respectively. A moderate root growth, i.e.,109.09% in *G harbaceum*, was found.

In most of the species, compared to shoot growth, root growth was higher. This observation proposes that the plants have prioritized the root expansion for nutrients and water absorption before shoot elongation. The highest shoot growth in *T aestivum* is exceptional, demonstrating the favourable conditions like nutrient availability, genetic factors, or the effects of a specific treatment. *C arietinum's*root growth suggests the adaptation strategy for water and nutrient uptake, which is a common mechanism in legumes. A balanced growth in *Zea mays* explains the balanced biomass allocation.

The overall observation depicted in graph no. 6 suggests that plants with high root growth may be optimising for nutrient gain, while those with higher shoot growth may be focusing on photosynthesis and biomass growth.

Discussion:

Phytotoxicity analysis is important for evaluating the environmental safety of treated dye wastewater before its release into the natural water ecosystem or use for agricultural purposes. The toxicity of textile dye wastewater is mainly due to its complex chemical structures, robust nature, and potential breakdown to form toxic intermediates (Verma *et al*., 2020) [17]. Therefore, it is important to assess the impact of treated effluent on plant growth to determine its environmental sustainability.

Biodegradation of azo-dyes by actinomycetes and other microbes often results in the formation of non-toxic or less toxic metabolites. It has been reported that the actinomycete species like *Streptomyces* and *Nocardia* can produce enzymes like azoreductase and laccase, which help in the breakdown of dyes into simpler compounds with less or no toxicity (Bhatt *et al*., 2021) [2]. Some of the studies have confirmed that plants, when cultivated with treated dye wastewater, exhibit usual germination rates and growth patterns compared to those exposed to untreated effluents (Singh *et al*., 2023) [16].

Kumar *et al*. (2021) [10] have reported the significant inhibition of seed germination and root elongation due to the presence of toxic dye intermediates. Even partially degraded compounds also bear some toxicity, depending on the efficiency of biodegradation pathways. The fully biodegraded wastewater has shown negligible poisonous effects, making it suitable for cropping or safe discharge into the environment (Patel *et al*., 2022).

Solomon et. al. (2024), in their research, have reported that treated wastewatercan enhance plant growth due to the presence of nutrients like nitrogen and phosphorus. The study was based on cherry tomato (Solanum lycopersicum) and cabbage (Brassica oleracea), where they found that irrigation with treated wastewater promoted crop growth instead of adversely affecting the photosynthetic efficiency or plant vitality.

Conclusion

The competent TB14 isolate of the actinomycetes culture involved in the current study degraded the toxic textile azo dye. In comparison to the other water samples, the treated wastewater has shown positive results in all the crop plants' growth regarding both the root and shoot length. This indicates the possible use of the treated wastewater for cropping, gardening, or general vegetation purposes.

Due to water scarcity in drought regions, it is an absolute necessity to make wastewater reusable for cropping purposes. The direct use of wastewater from industries such as the textile industry is unacceptable for crop development because of its harmful effects. These effects can be reversed by the use of actinomycetes capable of removing the pollutants from the environment.

Fig 1: Root and shoot analysis of Chickpeas with tap water, distilled water, media with dye, media with treated dye

Fig 2: Root and shoot analysis of cotton with tap water, distilled water, media with dye, and media with treated dye

Fig 3: Root and shoot analysis of maize with tap water, distilled water, media with dye, and media with treated dye

Fig 4: Root and shoot analysis of Moong with tap water, distilled water, media with dye, media with treated dye

Fig 5: Root and shoot analysis of wheat with tap water, distilled water, media with dye, and media with treated dye

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