

Comparative assessment of phytotoxicity in untreated and biotreated Industrial dye effluents using selected agricultural crops

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ABSTRACT

Heavy metals and synthetic dyes have been widely released into agricultural landscapes as a result of industrialization, especially in areas that border industrial zones. Plant physiological and biochemical processes are negatively impacted by these pollutants, which lowers production and may be harmful to human health due to bioaccumulation in the food chain. A major environmental problem is the release of inadequately treated industrial effluents into water bodies and, consequently, agricultural soils, which leads to soil degradation and lower crop yields. In the present investigation, the phytotoxic effects of an industrial dye and its metabolites were systematically evaluated using *Cicer arietinum*, *Lens culinaris*, *Cajanus cajan*, and *Triticum* seeds as model crops. Germination and early seedling growth parameters—namely germination percentage, phytotoxicity percentage, seedling vigor index, relative toxicity, relative seed germination, relative root growth, germination index, and germination rate index—were determined following established protocols reported in previous literature. These parameters served as reliable indicators of dye-induced stress during early plant development. The study aimed to assess and compare the relative phytotoxicity of untreated and biologically treated dye effluents to evaluate the potential of biotreatment in mitigating dye toxicity and restoring environmental safety for agricultural applications.

Keywords: Phytotoxicity; Industrial effluents; Dye degradation; Biotreatment; Seed germination; Environmental remediation; Crop development, Phytotoxicity, *Cicer arietinum*, *Lens culinaris*, *Cajanus cajan*, *Triticum*, seed germination, vigour index, relative toxicity, relative root growth, germination index

INTRODUCTION

Natural water pollution from industrial waste water is increasing due to the nation's industries' rapid growth. Surface and ground water resources can be harmed by the various pollutants that industries release, some of which contain chemical elements in undesired concentrations. The decreasing quality of the environment has become a significant problem in the modern era (Noel and Rajan 2015). A major worldwide issue, pollution is getting worse every day as a result of industrial activity (Kumar *et al.*, 2022). Any country's economic development is greatly affected by industrialization. The result of this was the massive industrial activity that took place all across the planet. Pollution is the main drawback of industrialization, despite its many favorable qualities (Noel and Rajan, 2015). Over 10,000 dyes are commercially accessible worldwide, and over 0.7 million tons of dyes, including basic, reactive, acidic, and diazoic

dyes, are produced annually. An estimated 200,000 tonnes of unbound azo dyes are lost annually as a result of poor dyeing methods during the dyeing and finishing processes in the effluents. Approximately 15% of dye-containing wastewater is released into the environment worldwide without treatment (Kusumlata *et al.*, 2024). These industries are the main producers of effluents, which ultimately leads to a significant production of wastewater (Rahman *et al.*, 2018). Since a lot of synthetic dyes that are resistant to traditional wastewater treatments are released into the environment every day, the textile industry presents environmental management challenges even though it contributes significantly to the economies of many nations by offering a wide variety of colored fabrics for marketing (De Oliveira *et al.*, 2018). A substantial quantity of untreated dye materials are present in textile effluents, which are discharged as sludge into nearby aquatic environments. This sludge's ultimate disposal

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is still difficult, and reducing its negative effects on the ecosystem requires an ecotoxicological evaluation (Ullah *et al.*, 2014). According to international standards for aqueous effluents, water treatment helped reduce wastewater's toxicity before it was released into the environment (WHO 2012). Textile dyes are among the many synthetic chemical compounds that are resistant to the typical biological treatment now in use (Zeghioud *et al.*, 2021). Determining its toxicity for species that belong to different trophic levels is important in order to acquire more ecotoxicological knowledge and, ultimately, establish its safe threshold for environmental protection. Reactive Red 120 has already been shown to exhibit species-specific toxicity, supporting the idea that a multitrophic level eco-tests method is the most reliable option to estimate the ecotoxic effects caused by textile dyes. The decolorization of textile dye effluent and the toxicity of the parent chemicals and byproducts to aquatic life have been the main topics of earlier studies on the environmental consequences of dyes. The specific effects of textile dyes on root elongation and seed germination, however, are not well documented (DeOliveira *et al.*, 2018). According to some sources, farmers use water from rivers and streams that consume wastewater from various dyeing factories to irrigate their gardens. Because it contains nutrients that might be used in farming, they view the wastewater as a resource that can be connected for beneficial application. For a very long period, this conduct has continued unchecked. When a higher volume of dye effluent was mixed with irrigation water, they detected some phytotoxic effects. In recent years, using industrial effluents for irrigation has become a significant wastewater utilization method, using the significant amounts of N, P, K, and Ca present along with the other essential nutrients (Noel and Rajan, 2015). According to Rodosevich *et al.* (1997), seed germination is an essential process since it ensures reproduction, regulates the growth of the plant population, and also likely increases harvest efficiency. Bioassays for seed germination and early plant development are the most well-known methods for evaluating phytotoxicity. These kinds of works have been performed in various countries across the world. Previous studies found that even

extremely diluted industrial effluent negatively decreased seed germination when working with *Cicer arietinum*. Additionally, chemicals collected from the growing media intoxicate live tissues, resulting in phytotoxicity. The species, kinds, and quantities of toxic chemicals in the effluent determine the negative impact of the pollutants on plants (Rahman *et al.*, 2018). Reactive Red 120 (RR120), a dye that is commonly utilized in the textile industry, presents a risk to aquatic life because of its low biodegradability. According to a prior publication, the toxicity effect of RR120 dye on the plant species *A. cepa* demonstrated that the dye causes oxidative stress, which in effect damages the root cells. On the other hand, RR120 dye's biodegraded metabolites are less toxic to plant species (Anastopoulos *et al.*, 2024)

MATERIALS AND METHODS

2.1 Dye and chemicals

The study applied the textile RR120 dye ($C_{44}H_{30}N_{14}O_{20}S_6$, double azo dye, mol. wt.: 1469.98 g/mol). The textile is where the RR120 is most frequently utilized. However, every reagent and media component utilized in the studies was of the highest purity and analytical quality (Sigma-Aldrich Chemicals Pvt. Ltd).

2.2 Bacterial strain

The following bacterial cultures were used in the study: *L. capsici* (accession no: 13606, *A. baumannii*; accession no: 13438, *A. faecalis subsp. Phenolicus*; accession no: 13436, *P. aeruginosa*; accession no: 13437).

2.3 Phytotoxicity assay

A phytotoxicity experiment was conducted in the Department of Biotechnology's growth chamber at GGU Bilaspur in Chhattisgarh, India. The parent and extracted RR120 dyemetabolites degraded by *L. capsici*, *A. baumannii*, *A. faecalis subsp. Phenolicus* and *P. aeruginosa* bacterial strains toxicity was evaluated using *Cicer arietinum*, *Lens culinaris*, *Cajanus cajan* and *Triticum* seeds (fig.1) sterilized for five minutes with 0.1% HgCl₂ (w/v). Three groups were subjected to the assay: one the parent dye, water, and the degraded dye metabolite. Seeds were deeped (Fig.2) all three sample for

12 hours, which was then put through a phytotoxicity test. On the other hand, the seeds and irrigation were treated with distilled water as a control. All tests were applied to the seeds, and they maintained at room temperature during each treatment. After 48 hours of treatment, the rate of seed germination was assessed. The seeds that had sprouted were moved to a plastic container that had cotton at the bottom for a substrate. Subsequently, they were subjected to the RR 120 dye and its breakdown products. The

assay was carried out for seven days while the observation maintained. The seedlings (fig.3) were taken out for additional examination on the seventh day of the experiment. To see any notable changes, the parameters, including the percentage of seed germination, phytotoxicity %, seedling vigor index, relative toxicity, relative seed germination, relative root growth, germination index, germination rate index, required to be noted (Kalyani *et al.* 2009; Rao and Prasad 2014).

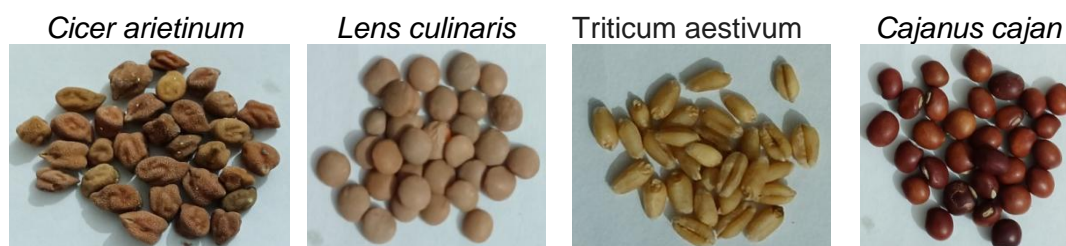


Fig.1: Used seeds for phytotoxicity study

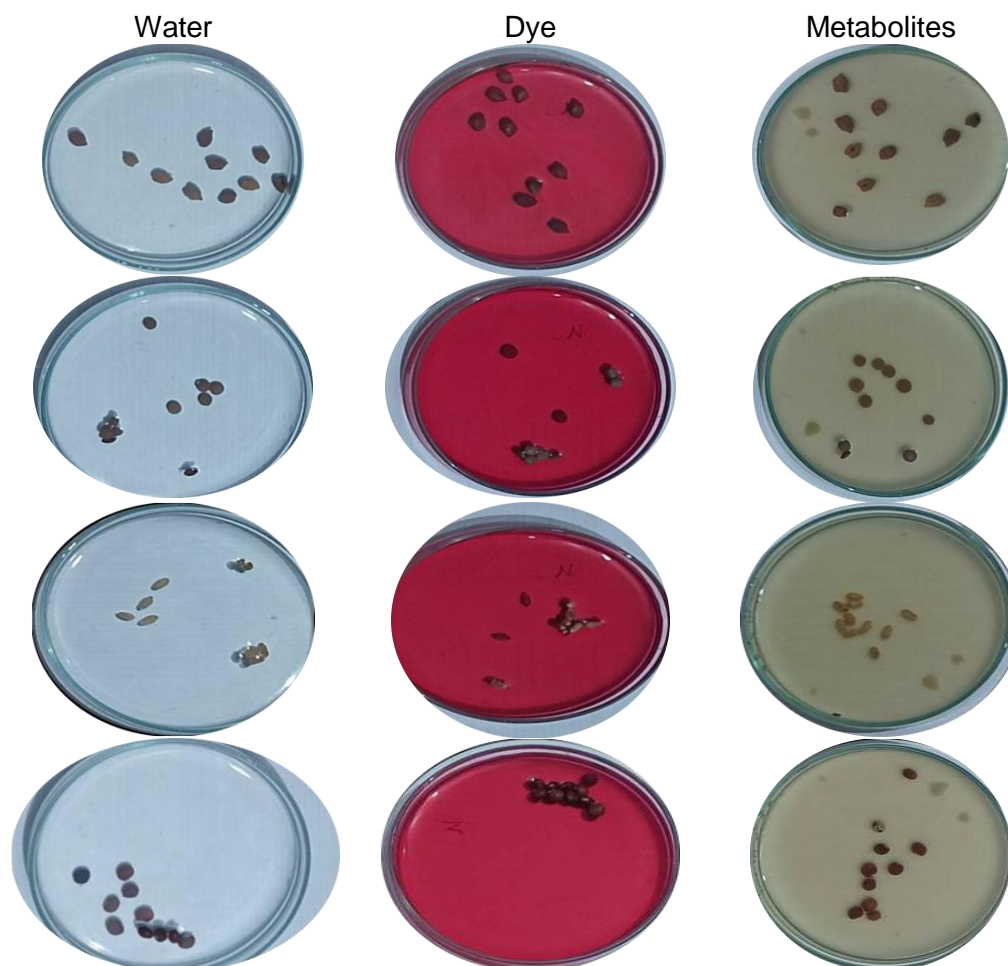


Fig. 2: Deeped seeds in water, dye solution and dye metabolites samples



Fig. 3: Germinated seeds

Parameters studied: The characteristics mentioned below were studied.

a) Seed germination %: The following formula was used to calculate the percentage of seed germination:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds}} \times 100$$

b). Phytotoxicity %: The following formula was used to calculate the percentage of phytotoxicity:

$$\text{Phytotoxicity \%} = \frac{\text{Mean root length in control} - \text{mean root length in sample}}{\text{Mean root length in sample}} \times 100$$

c). Vigour index: The Abdul-Baki and Anderson (1973) formula was used to calculate the seedling vigor index.

$$\text{Vigour index} = \text{Germination \%} \times \text{Length of seedling}$$

d). Relative toxicity %: The following formula was used to determine the relative toxicity of dye effluent on germination compared to control.

$$\text{Relative Toxicity \%} = \frac{x-y}{x} \times 100$$

Where, x = Germination percentage in control at a particular hour of incubation; y = Germination percentage in the presence of effluent at the same hour of incubation.

e). Relative seed germination: The number of seeds that germinated in the sample and the number of seeds that germinated in the control were counted for each experimental set following the seven-day experiment. Using Equation, the relative seed germination (RSG) was calculated.

$$\text{RSG \%} = \frac{\text{Number of seeds germinated in compounds}}{\text{Number of seeds germinated in control}} \times 100$$

f). Relative root growth%: For every experimental set, the root lengths of the sample and the control were measured. The main root's end to the hypocotyl's base was used to measure the root's length. The relative root growth (RRG) was calculated using Equation to get its percentage.

$$\text{RRG \%} = \frac{\text{Mean root of length in compounds}}{\text{Mean root length in control}} \times 100$$

g). Germination index: It was calculated according to with formal seed analysis criteria and represents the estimated germination rate of seedlings.

$$\text{GI\%} = \frac{\text{RSG} \times \text{RRG}}{100}$$

h). Germination rate index: The germination rate index was calculated by dividing the

germination index by the germination percentage for each treatment and replication.

$$\text{GRI} = \frac{\text{Germination index}}{\text{Germination \%}}$$

STATISTICAL ANALYSIS

The results are mean \pm standard

deviation, and all research experiments were carried out in triplicate ($n = 3$). Means and standard deviations were used to show the data. MS software was used for each statistical procedure.

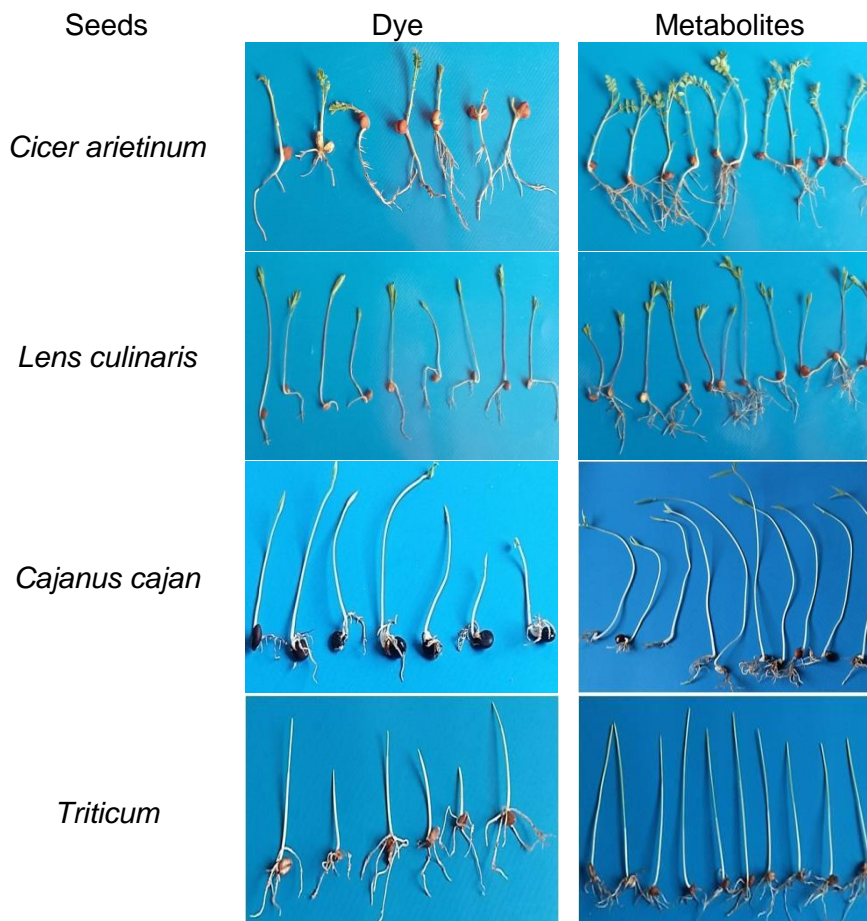


Fig. 4: Affected the size of the plumule and roots in the germinated seeds

RESULTS

Phytotoxicity effect of degraded and non degraded dye

The dye toxicity was proven by the 70%, 90%, 70% and 60% germination rate of *Cicer arietinum*, *Lens culinaris*, *Cajanus cajan* and *Triticum* seeds exposed to the parent dye without any treatment. In comparison to the treated seeds, which had plumules that were longer, the untreated seeds' plumules were significantly smaller (fig.4 table 1). However, after treatments, the toxicity of the parent dye was reduced because the all seeds treated with

treated dye metabolites samples showed no evidence of toxicity and a 100% germination rate (fig. 5a). Furthermore, there were no harmful effects from treating the seeds with distilled water; they germinated 100% of the time and developed normally. Additionally, the size of the plumule and the total number of roots in the germinated seeds were significantly reduced by the untreated dye solution. Our research showed that the untreated dye solution had a significant phytotoxicity percentage of seeds. Comparing the treated dye, the phytotoxicity percentage (fig. 5b) was decreased.

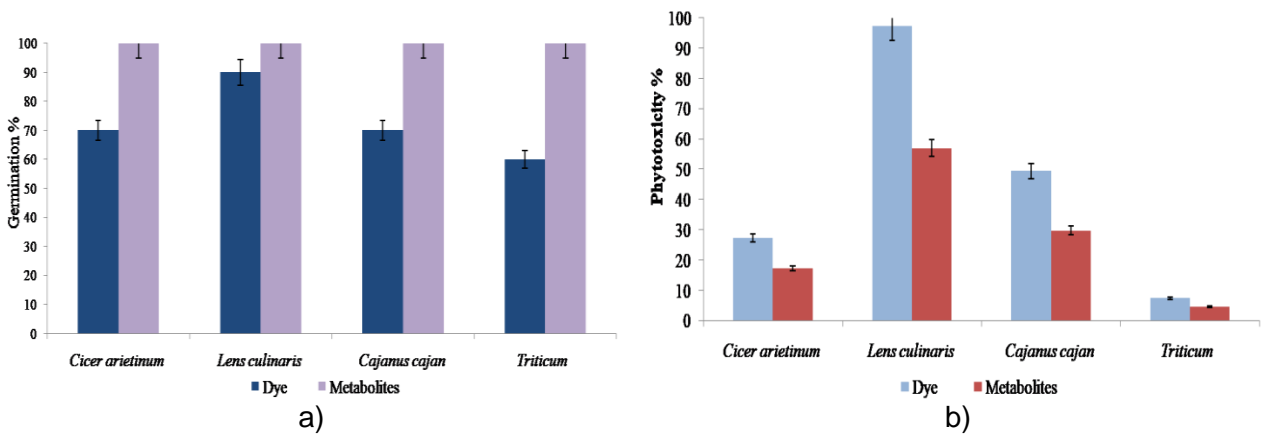


Fig. 5: Analysed germination % (a) and phytotoxicity % (b) for dye and their metabolites

The *Cicer arietinum*, *Lens culinaris*, *Cajanus cajan* and *Triticum* plants in the untreated dye sample had vigour index (fig. 6a), relative toxicity % (fig. 6b), RSG% (fig. 6c) and RRG% (fig. 6d) of 228.9, 363.6, 270.2, 280.2, 30%, 10%, 30%, 40%, 70%, 90%, 70%, 60%, 78.51%, 50.67%, 66.93% and 93.05% respectively. Similar VI, RSG% and RRG% were

found in plants in the treated dye sample, which were 861, 747, 166, 385, 100%, 100%, 100%, 85.27, 63.68, 77.09, 95.53 and RT percentages was 0% respectively. The results showed that toxicity had a significant effect on the vigour index and relative toxicity % of both treated and untreated samples, which inhibited plants growth.

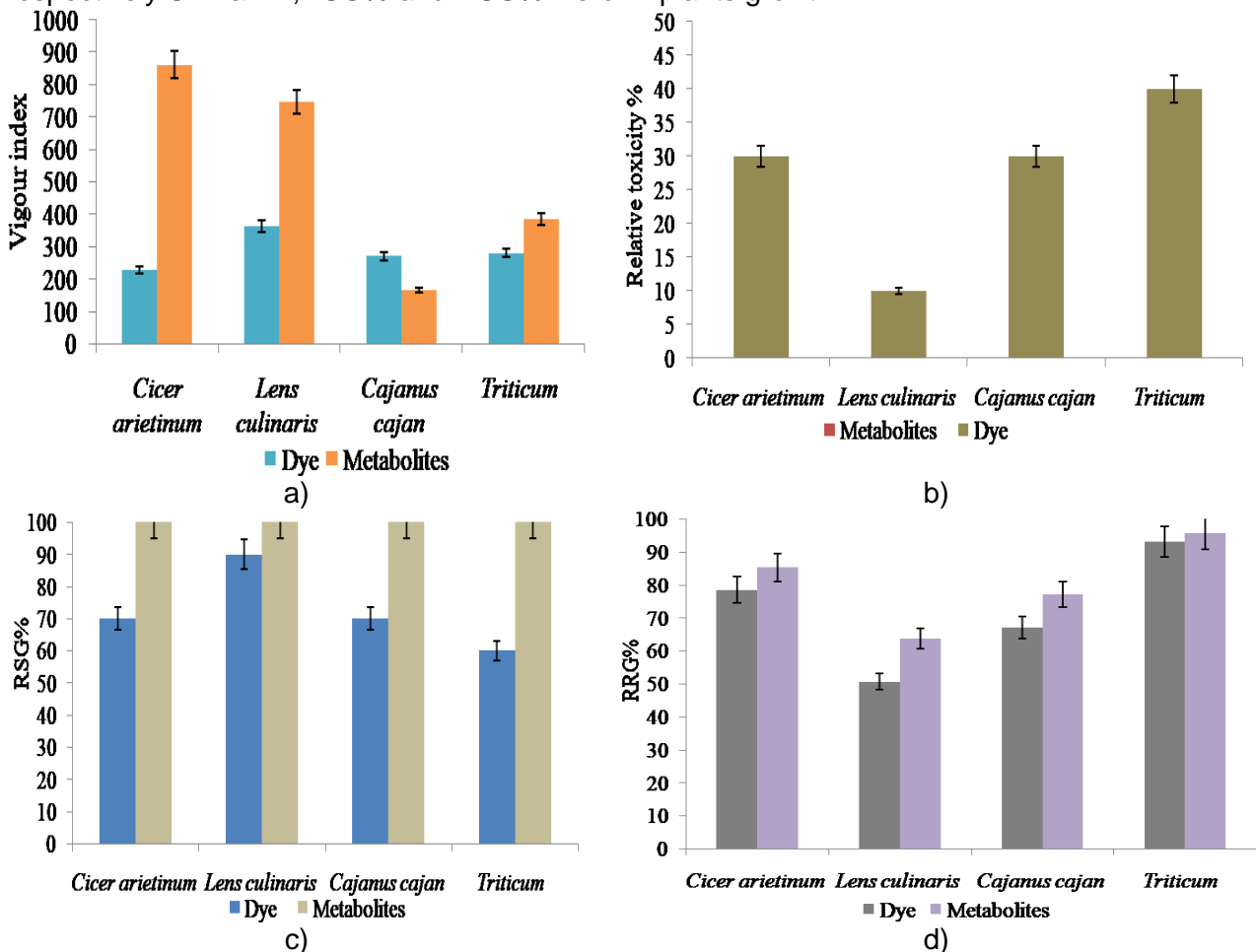
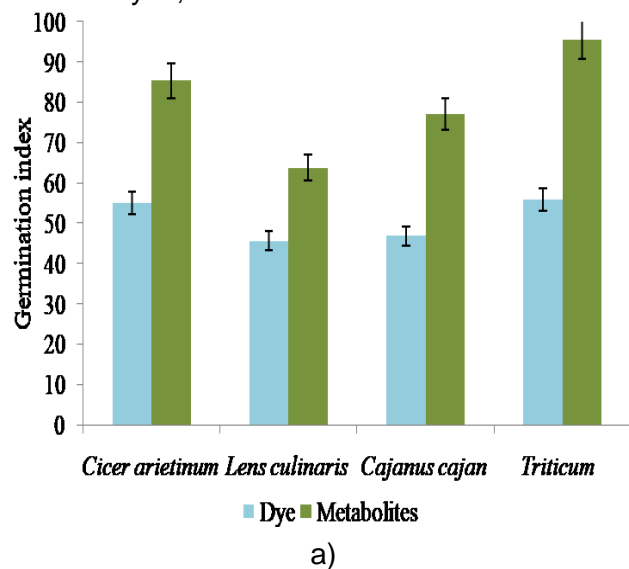


Fig. 6: Effect of dye and their metabolites on the a) vigour index, b) relative toxicity %, c) RSG% and d) RRG% of seeds

These findings are further confirmed by the germination index and germination rate index data, which indicate lower values when compared to the treated dye. In these crops, untreated dyes are more phytotoxic than treated dyes, and the decrease in GI and GRI



values with parent synthetic dyes was greater than that with treated dye metabolites, indicating that the first kind is comparatively more susceptible to the toxic action of the synthetic dyes. The findings in (fig. 7a and b) showed.

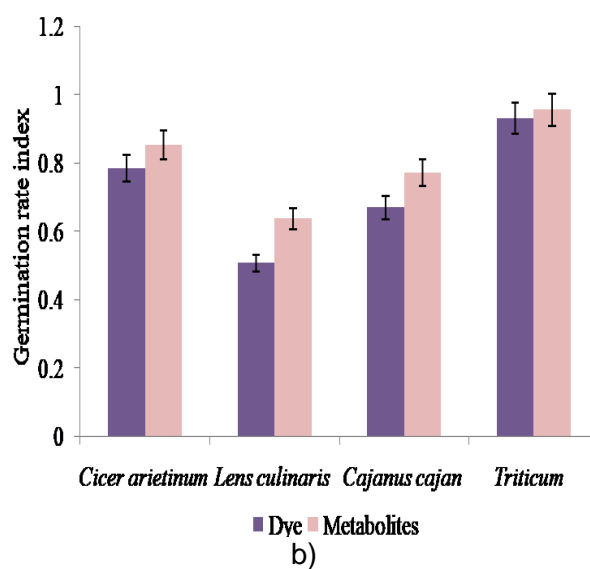


Fig. 7: Effect of dye and their metabolites on the a) germination index, b) germination rate index

CONCLUSION

In conclusion of our study, the bacterial strains *L. capsici*, *A. baumannii*, *A. faecalis subsp. Phenolicus*, *P. aeruginosa* showed remarkable efficacy in degrading the RR120 dye within 12 hours. Our previous papers reported GCMS and FTIR analyses reveal variations of the original dye and its metabolites. Additionally, phyto-toxicity tests conducted were used *Cicer arietinum*, *Lens culinaris*, *Cajanus cajan* and *Triticum* crop seeds. In a phytotoxicity test, 100% seed germination and healthy plant growth confirmed that bacterial strains effectively altered RR120 into non-toxic byproducts. The seeds treated with metabolite products had a better rate of germination. The phytotoxicity studies showed that the breakdown products were less hazardous than untreated dye. Therefore, we firmly encourage the use of bacteria for textile

pollutant cleanup and detoxification. Additionally, recycling dye effluent that has been degraded and detoxified for use in crop irrigation will be beneficial. Since the toxicity of dyes is significantly reduced, biodegradation is more environmentally friendly than conventional methods.

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Author contributions- Kusumlata performed of the study, writing, Ashish Kumar reviewed and analyzed the data.

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