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Original Article

Evaluating the Effects of *Amorphophallus paeoniifolius* Enzyme Extract on Seed Growth Parameters in Selected Crops Exposed to Treated Textile Dyes and Wastewater

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Abstract

Dyes and industrial effluents often contain toxic substances that can hinder seed germination and growth by affecting water uptake and nutrient absorption therefore understanding the impact of these pollutants on plants is crucial. The current study aims to test the effects of dye and effluent on the germination and growth of seeds of *Vigna radiata, Cicer arientinum*, and *Triticum aestivum*, with and without the addition of crude enzyme from *Amorphophallus paeoniifolius.* The study focuses on four key parameters i.e. germination percentage, root length and shoot length, vigour index, fresh and dry weight of seed. Crude enzyme from *A. paeoniifolius* positively impacts seed germination, boost seedling development (Shoot and Root lengths) and (fresh and dry weight) of *Vigna radiata, Cicer arientinum*, and *Triticum aestivum*. Untreated seed germination of *Vigna radiate* was between 40-70%, *Cicerarientinum* was between 20-70% and *Triticum aestivum* was 40-60% and after treatment with the enzyme seed germination percentage was increased between 60-90%,50-80 %, 60-90% respectively. Among all three plants *Vigna radiata* has highest seed germination percentage, maximum shoot length, root length and vigour index when treated with *A. paeoniifolius.* Therefore, seed germination and seedling development in both monocot and dicot plants concludes that treated water by *A. paeoniifolius* can be used to meet the water requirements for irrigation purpose but further studies are needed to test toxicity impacts under natural soil environment and on human through food chain.

Keywords: *Amorphophallus paeoniifolius, Crude Enzyme, Dyes, Seed Germination, Textile Effluent*

Introduction

Most pollution in the world today may be traced back to countries whose economies grew too quickly and without proper planning. Each industry is related to an output of various pollutants (Rahman *et al*., 2018). As there is a greater need for textiles, the textile industry has become a major industrial water user and a huge industrial wastewater generator, particularly in countries like India. Aquatic life, vegetation, and people are all at risk when factories dump sewage into rivers and streams without proper treatment. An excessive amount of effluent has a negative impact on the soil and can seriously retard crop development.

Industrial effluents provide the greatest risk to both water and soil quality (Rahman *et al*., 2019). Dyes used in the dying and printing industries are a major source of toxicity and aesthetic degradation in wastewater. Dye and bleaching processes in the textile industry produce wastewater that contains a wide variety of pollutants, including organo-chloride-based waste and heavy metals. These waste waters contain toxic chemicals and minerals that have a wide range of negative effects on plant life and

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soil (Hussain *et al*., 2013; Pandey *et al*., 2020). As these harmful substances in effluent accumulated in the cells of living beings, it led to a decrease in cell activities, a slowing of growth, numerous deficiencies, and illnesses (Rana & Kumar, 2017).

In industrial areas, groundwater is being rapidly polluted by industrial effluents. The industrial effluents produced lead to modifications in physiochemical and biological characteristics of the environment (Kathirvel, 2012). Plant growth, the germination process, and the development of seedlings will all be stunted by the presence of heavy metals and harmful substances. Increases in effluent concentration are detrimental to agricultural productivity (Shahena *et al*., 2021). The use of such wastewater in irrigation systems surely gives some nutrients to boost the fertility of soil, but it also deposits toxicants that modify soil characteristics in the long run. Reduced energy availability through anaerobic respiration slows seedling development when dissolved oxygen levels are low due to a high concentration of solids in the effluent (Shekhawat *et al*., 2021; Chanikya *et al*., 2021). There are major issues caused by the careless and reckless dumping of textile effluents into the environment. When exposed to effluent at a lower concentration, seedling growth is stimulated, and the plant matures more quickly. Increased particles in the effluent may be to blame for the decreased germination rate seen at greater effluent and dye concentrations since they alter the osmotic connection between the seed and the water (Fiaz *et al*., 2023). Organic compound metals and salts found in wastewater dye have an impact on the pH, TSS, COD, BOD, and color of the water (Anjali *et al*., 2022). Recently, there has been a lot of research on the use of plant-source enzymes to detoxify different textile effluents and dyes. White rot fungal liginolytic enzymes, such as laccase, MnP, and LiP, are essential for breaking down textile colours. To effectively cleanse wastewater, several colours and enzymatic mechanisms are essential (Kumar *et al*., 2024).

Phytoremediation is an eco-friendly method by which wastewater can be treated to reduce various contaminants that would otherwise be carried into the environment. A preliminary study by our group was done on the various wastewater parameters like BOD, COD, pH, *etc.* of various dyes and effluents used in this study by *A. paeonniifolius* crude enzyme extract (Anjali and Singh, 2022; Anjali *et al*., 2023).

A. paeoniifolius, popularly known as Jimikand, is a frequently underutilised plant that offers a number of health advantages (Singh & Wadhwa., 2014). The *Amorphophallus paeoniifolius* (Elephant-foot yam) is recommended as a cure by all three of the significant Indian medicinal systems (Ayurveda, Siddha, and Unani) (Raji *et al*., 2019). Various pollutants may be decolored and broken down by enzymes obtained from plant sources. *Amorphophallus paeoniifolius* is a cheap and accessible source of PPO enzymes identified by our research group earlier (Singh & Wadhwa, 2017).

Many enzymes, including PPO and peroxidase, can be used to clean wastewater. Seed germination studies of various plants like *Vignaradiata* and *Cicer arientum* using industrial effluents have been reported previously (Nawaz *et al*., 2006; Rohit *et al*., 2013).

The corm of *A. paeoniifolius* contains various enzymes (Singh *et al.,* 2015; Singh & Wadhwa, 2017). When compared to expensive chemicals, crude enzymes are a much more affordable option and don't require special equipment. The processed dyes and wastewater are more clean and less contaminated after being treated with crude enzyme from *A. paeoniifolius*. Less contaminated water has a direct impact on the length of the roots and shoots as well as the growth of seedlings. Seed germination and seedling growth rates gradually declined with increasing effluent concentrations, whereas the lower concentration of effluent and treated dyes showed high germination percentage, plumule, and radicle length (Kurmi *et al*., 2023).

Treated water of textile industry dyes and effluents from enzymes of underutilised *A. paeoniifolius corm may* be used for irrigation, which will not only help to utilise wastewater but will also increase the soil fertility and lead to high yielding of crop production. Therefore, the present study was designed to test the effects of various dyes and effluents on the seed germination and growth parameters of *Vigna radiata, Cicer arientinum*, and *Triticum aestivum*, with and without the addition of crude enzymes from *A. paeoniifolius*. The main objective of this research is to check the impact of various dyes and wastewater on monocot and dicot plant growth parameters by using *Amorphophallus paeoniifolius* crude enzyme extract. There is no study reported so far where the crude enzyme from *A. paeoniifolius* is used for treatment of dyes and effluent.

Material and Methods

Plant material and chemical

Amorphophallus paeoniifolius corms were purchased from the local market in Ghaziabad, and the seeds of dicot Vigna radiata, Cicer arientinum seeds, and monocot Triticum aestivum were procured from the School of Agriculture, Galgotias University. Acetone, the Biuret reagent, Sodium azide, Sodium chloride, Ascorbic acid, Citric acid, CaCl2, zinc oxide, and EDTA were procured from (Merck USA). All reagents used were of analytical grade. The textile dyes Red CE, Yellow CE, Blue CE, Neavy Blue CE, and untreated effluent were obtained from Sky Lark, a textile industry in Surajpur, Greater Noida. All samples were collected in the month of September 2022. The other chemicals and reagents utilised were all of the analytical quality and weren't further purified before use.

Preparation of Effluent sample

The dyes collected for the study are Red CE, Yellow CE, Blue CE, and Navy Blue CE.01 % dye solution was prepared for each dye. Each 67.5 ml dye and effluent were incubated with 7.5 ml crude enzyme from *A. paeoniifolius* extracted in phosphate buffer, pH 7.0, at 37 °C as "per the method by Kagalkar *et al*. (2009). The samples were collected in sterile, wide-mouth plastic vials and maintained in the refrigerator at 4^0 C to preserve their integrity. Solutions with various dyes and effluent were created to assess the effects of dyeing effluent on seeds of *Vigna radiata*, *Cicer arientinum*, and *Triticum aestivum*. Enzymatic activity of the unprocessed and partly purified enzyme was estimated as residual activity percentage (Onsa *et al*., 2000; Zocca *et al*., 2008; Singh & Wadhwa, 2017; Laad *et al*., 2020).

Treatment Level:

T- Treated

T1- Red CE: Crude enzyme from *A. paeoniifolius* (67.5ml +7.5ml= 75ml)

T2- Yellow CE: Crude enzyme from *A. paeoniifolius* (67.5ml+7.5ml= 75ml)

T3- Blue CE: Crude enzyme from *A. paeoniifolius* (67.5ml+7.5ml= 75ml)

T4- Navy-Blue CE: Crude enzyme from *A. paeoniifolius* (67.5ml+7.5ml= 75ml)

T5- Effluent: Crude enzyme from *A. paeoniifolius* (67.5ml+7.5ml= 75ml)

C- Control- (Distilled water - 75ml)

Treatment Level:(Without Crude enzyme)

UT-Untreated

UT1- Red CE (75ml)

UT2- Yellow CE (75ml)

UT3- Blue CE (75ml)

UT4- Navy-Blue CE (75ml)

UT5- Effluent (75ml)

C- Control- (Distilled water - 75ml)

During the germination experiments, seeds were sterilised using a mercuric chloride ($HgCl_2$) solution of 0.1 percent w/v for 5 minutes to kill any remaining bacteria and then rinsed three times with sterile distilled water. Ten uniformly sized seeds of *Vigna radiata*, *Cicer arientinum*, and *Triticum aestivum* were planted in sterilised glass petri dishes with filter paper discs at the bottom. The study was performed in triplicate for 7 days. Distilled water served as a control, while treated and untreated dye and effluent with crude enzyme from *A. paeoniifolius* were applied to the filter discs, respectively. At room temperature, the petri plates were incubated. For six days in a row, 5 ml of each dilution was sprayed. Daily reports on the progress of the seedlings were kept. All the tests were performed in triplicate. On the seventh day, growth metrics and germination rate were examined.

Germination percentage- For this purpose, we utilised the method for estimating germination rate provided by Rehman (Rehman *et al*., 1998).

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

Root and Shoot Length-Root and shoot lengths (radicle and plumule of seedlings) were measured by using standard centimetres scale.

Vigour index- Vigour index was determined using a method proposed by (Abdul-Baki *et al.,*1973).

Vigour index = % Germination X (shoot length* + root length*)

(* represents the length of roots and shoots, in centimetres).

Fresh and Dry Weight of seeds – Fresh weight was determined by weighing 10 seeds from each treatment and non-treatment, and dry weight was determined by drying the seeds at 80 °C for 24 hours. The measurements for both the wet and dry weights were taken in milligrams (Nedeva *et al*., 1999).

Statistical Analysis

Statistical significance of the variables was evaluated through a t-test using graph pad prism 9. The comparison test as applied for mean comparison values is represented as mean ± SD of triplicate measurement. A student's t-test was applied to determine significance, and a p-value of ≤ 0.05 was a significant value.

Result

All three plants were checked for the mentioned parameters with treated and untreated dyes and effluent. Table 1 compares the germination percentage, vigour index, shoot length, root length, fresh weight, and dry weight of *Vigna radiata* grown without the addition of crude enzyme with various dyes and effluent.

A study of *Vigna radiata* seed germination percentages reveals that distilled water seedlings (control) had a 100% germination rate, while untreated seeds without the *A. paeoniifolius* crude enzyme have a germination percentage between 30% - 70% (Table 1 and Figure 1 A), displays the greatest reductions in seed germination reported in Navy Blue CE dye (UT4) and effluent (UT5) concentrations were 30% and 40%, respectively.

The vigour index of *Vigna radiata* seedlings grown in distilled water (control) was 1450, whereas the vigour index of seedlings grown without the *A. paeoniifolius* crude enzyme was reduced to 300 to 875. (Table 1 and Figure 1 A).

Vigna radiata seedlings grown in distilled water (control) had a shoot length of 6.5 cm, whereas those grown from seeds not treated with the *A. paeoniifolius* crude enzyme were shorter by between 1.1 and 2.5 cm. The highest reduction at Navy-Blue CE dye (UT4) without the *A. paeoniifolius* crude enzyme was determined to be 2.5 cm (Table 1 and Figure 1 B).

Table 1: Effect of dye and effluent without Crude enzyme from *A. paeoniifolius* on some growth parameters of *Vigna radiate*

Un-treatment	**Seed germination Percentage (7th days)**	**Vigour index (Mean±SD)**	**Seedling growth (14th days)**		**Weight**	
			Shoot length (cm.) Avg. (Mean±SD)	**Root length (cm) Avg. (Mean±SD)**	**Fresh weight (mg) (Mean±SD)**	**Dry weight (mg) (Mean±SD)**
UT1	60	750± 8.34	5.5±0.23	7±0.21	2.72±0.07	1.41±0.08
UT2	50	625± 9.67	5±0.19	7.5±0.28	2.81±0.13	1.54±0.06
UT3	70	875±9.13	5.5±0.17	7±0.39	2.12±0.09	1.65±0.06
UT4	30	300±9.12	4±0.27	6±0.13	2.63±0.08	1.53±0.12
UT5	40	476±10.89	5.4±0.34	6.5±0.18	2.51±0.13	1.32±0.08
C	100	1450±11.56	6.5±0.16	8±0.24	3.26±0.04	1.67±0.07

UT1- Red CE (75ml); UT2- Yellow CE (75ml); UT3- Blue CE (75ml); UT4- Navy Blue CE (75ml); UT5- Effluent (75ml); C- Control- (Distilled water - 75ml)

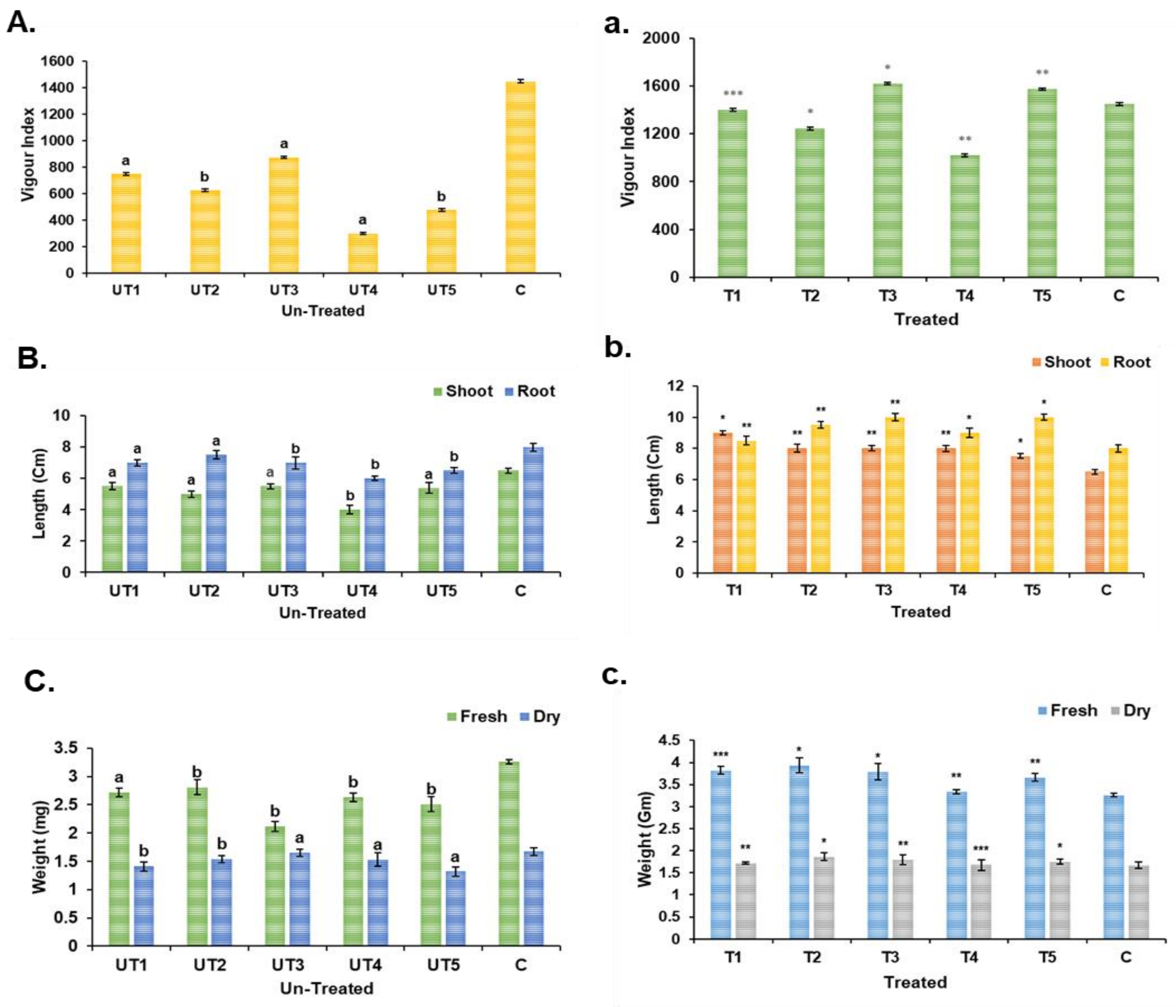

Figure 1- Bar-graph showing the impact of dying and effluent without and with Crude enzyme from *A. paeoniifolius* on growth parameters of *Vigna radiata* Seeds: **A.** Vigour index; **B.** Seedling growth; **C.** Weight; **a.**Vigour index; **b.** Seedling growth; **c.** Weight (UT1- Red CE; UT2- Yellow CE; UT3- Blue CE; UT4- Navy-Blue CE; UT5- Effluent; T1- Red CE; T2- Yellow CE; T3- Blue CE; T4- Navy-Blue CE; T5- Effluent; C- Control).The values are expressed as mean ± standard deviation (n = 3) and $^{a}p > 0.06$; $^{b}p > 0.05$; $^{*}p < 0.001$; $^{**}p < 0.004$; $^{***}p < 0.02$ compared with the control (**UT-** Untreated ,**T-**Treated).

The root length of *Vigna radiata* seedlings grown in distilled water (control) was 8 cm, whereas the root length of seedlings grown without the *A. paeoniifolius* crude enzyme was lowered by between 0.5 and 2 cm. The highest reduction at Navy Blue CE dye (UT4) without the *A. paeoniifolius* crude enzyme was determined to be 2 cm (Table 1 and Figure 1 B).

The fresh weight of *Vigna radiata* seedlings in distilled water (control) was 3.26 gm, whereas the fresh weight of seedlings untreated with the *A. paeoniifolius* crude enzyme was reduced by between 0.14 and 0.75 gm. The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 0.75 gm at effluent (UT5) (Table 1 and Figure 1 C).

Vigna radiata seedlings grew to 1.67 g in dry weight when cultivated in distilled water (control), but only 0.02 to 0.35 g in dry weight when not treated with the crude enzyme from *A. paeoniifolius.* The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 0.35 gm at effluent (UT5) (Table 1 and Figure 1 C).

Table 2 shows the results of a comparison of the germination percentage, vigour index, shoot length, root length, fresh weight, and dry weight of *Vigna radiata* cultivated in conditions with distilled water (control) with the *A. paeoniifolius* crude enzyme.

Table 2: Effect of dye and effluent treated with Crude enzyme from *A. paeoniifolius* on some growth parameters of *Vigna radiate*

Treatment	**Seed germination Percentage (7th days)**	**Vigour index (Mean±SD)**	**Seedling growth (14th days)**		**Weight**	
			Shoot length (cm.) Avg. (Mean±SD)	**Root length (cm) Avg. (Mean±SD)**	**Fresh weight (mg) (Mean±SD)**	**Dry weight (mg) (Mean±SD)**
T1	80	1400±13.24	9±0.14	8.5±0.26	3.82±0.09	1.72±0.03
T2	70	1246±12.97	8±0.26	9.5±0.21	3.93±0.17	1.86±0.09
T3	90	1620±10.2	8±0.18	10±0.23	3.79±0.18	1.79±0.11
T4	60	1020±11.42	8±0.19	9±0.31	3.34±0.05	1.68±0.12
T5	90	1575±9.79	7.5±0.15	10±0.19	3.66±0.09	1.75±0.06
C	100	1450±11.56	6.5±0.16	8±0.24	3.26±0.04	1.67±0.07

A study of *Vigna radiata* seed germination percentages reveal that distilled water seedlings (control) had a 100% germination percentage, while treated seeds with the *A. paeoniifolius* crude enzyme have a germination percentage between 60% and 90% lower than controls (Table 2). Germination percentage was increased when we were treating dyes and effluent with enzymes. The greatest reductions as compared to control 60% were reported in T4 (Navy-Blue CE dye).

The vigour index in untreated seedlings (control) of *Vigna radiata* was 1450, while the vigour index of seedlings treated with the *A. paeoniifolius* crude enzyme was increased 1620 in comparison to control. The vigour index of treatment level T1- Red CE; T2- Yellow CE; and T4- Navy-Blue CE with the crude enzyme of *A. paeoniifolius* were decreased 1400, 1246, and 1020 respectively. The maximum reduction was found 1020 in Navy-Blue CE dye (T4) as compared to control (Table 2 and Figure 1a).

Vigna radiata seedlings grown in distilled water (control) had a shoot length of 6.5 cm, whereas those grown from seeds treated with the *A. paeoniifolius* crude enzyme were increasing by between 1 and 2.5 cm. The highest increase in Red CE dye (T1) with the *A. paeoniifolius* crude enzyme was determined to be 2.5 cm (Table 2 and Figure 1 b).

The root length of *Vigna radiata* seedlings grown in distilled water (control) was 8 cm, whereas the root length of seedlings grown with the *A. paeoniifolius* crude enzyme was increasing by between 0.5 and 2 cm. The highest increasing in Blue CE (T3) and Effluent (T5) with the *A. paeoniifolius* crude enzyme was determined to be 2 cm, respectively (Table 2 and Figure 1 b).

The fresh weight of *Vigna radiata* seedlings in distilled water (control) was 3.26 gm, whereas the fresh weight of seedlings treated with the *A. paeoniifolius* crude enzyme was increasing by between 0.08 and

0.67 mg. The presence of the *A. paeoniifolius* crude enzyme resulted in a maximum increase of 0.67 mg in Yellow CE dye (T2) (Table 2 and Figure 1 c).

Vigna radiata seedlings grew to 1.67 g in dry weight when cultivated in distilled water (control), but only 0.02 to 0.19 mg in dry weight when not treated with the crude enzyme from *A. paeoniifolius*. The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 0.19 mg in Yellow CE dye (T2) (Table 2 and Figure 1 C). All the visual appearance of different dyes in *Vigna radiata* is shown in Fig. 2.

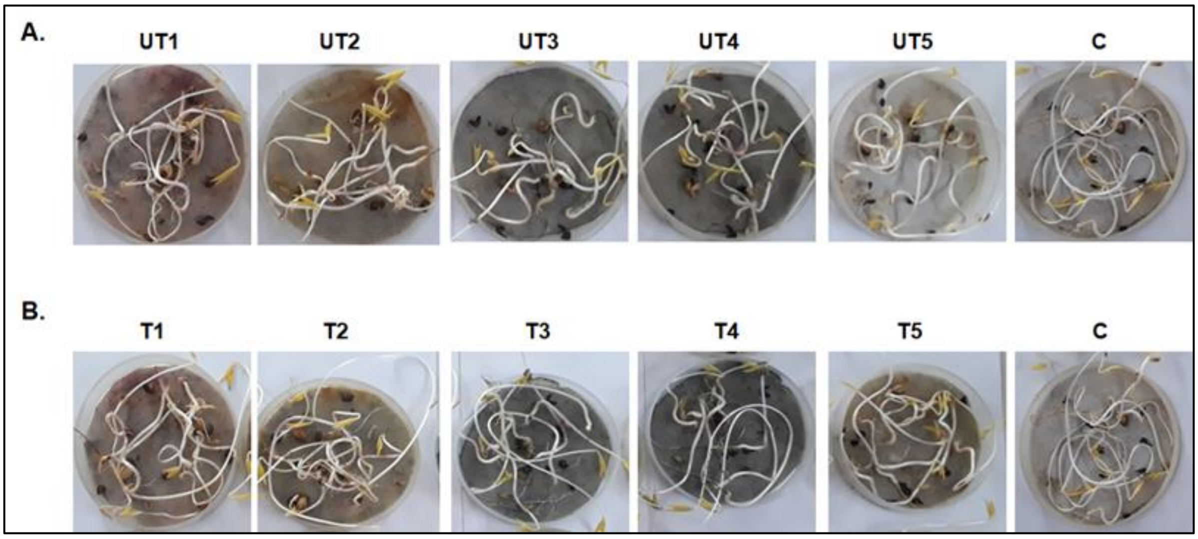

Figure 2: Seedling growth of *Vigna radiate* **A.** Without crude enzyme from *A. paeoniifolius* **B.** with Crude enzyme from *A. paeoniifolius*

Table 3 and Table 4 compare the parameters like germination percentage, vigour index, shoot length, root length, fresh weight, and dry weight of *Cicer arientinum* grown in different conditions with distilled water (control) and with all the samples, without crude enzyme, and with the *A. paeoniifolius* crude enzyme, respectively.

Table 3: Effect of dye and effluent without Crude enzyme from *A. paeoniifolius* on some growth parameters of *Cicer arientinum*

Un-treatment	**Seed germination Percentage (7th days)**	**Vigour index (Mean±SD)**	**Seedling growth (14th days)**		**Weight**	
			Shoot length (cm.) Avg. (Mean±SD)	**Root length (cm) Avg. (Mean±SD)**	**Fresh weight (mg) (Mean±SD)**	**Dry weight (mg) (Mean±SD)**
UT1	70	525±10.23	4.5±0.23	3±0.18	3.12±0.09	2.21±0.11
UT2	50	325±15.67	3.5±0.14	3±0.15	3.51±0.06	2.34±0.16
UT3	50	300±8.78	3±0.15	3±0.19	4.32±0.04	2.15±0.08
UT4	40	260±7.89	3.5±0.2	3±0.28	3.13±0.11	2.53±0.09
UT5	20	140±6.89	3.5±0.21	3.5±0.11	3.61±0.05	2.52±0.11
C	100	1240±9.45	5.5±0.18	6.9±0.16	4.96±0.1	2.87±0.12

A study of *Cicer arientinum* seed germination percentages reveal that distilled water seedlings (control) had a 100% germination rate, while untreated seeds without the *A. paeoniifolius* crude enzyme have a germination percentage between 20% and 70% lower than controls (Table 3). The greatest reductions reported in effluent (UT5), i.e., 20%.

The vigour index of *Cicer arientinum* seedlings grown in distilled water (control) was 1240, whereas the vigour index of seedlings grown without the *A. paeoniifolius* crude enzyme was reduced to 140 to 525. Among all the samples, the maximum vigour index was reduced to 140 in untreated effluent (UT5) in the absence of the crude enzyme from *A. paeoniifolius*, (Table 3 and Figure 3 A).

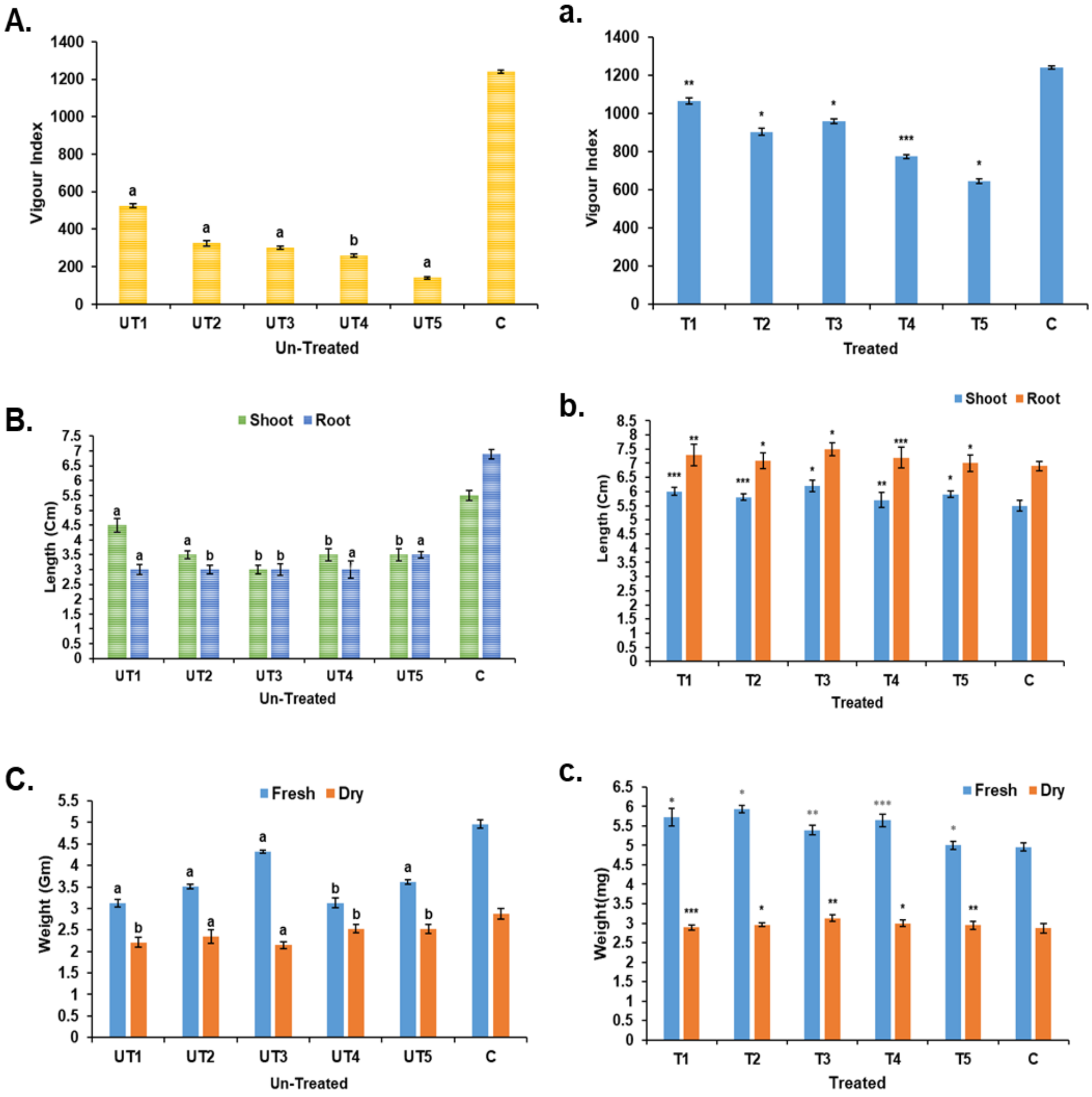

Figure 3- Bar-graph showing the impact of dying and effluent without and with Crude enzyme from *A. paeoniifolius* on growth parameters of *Cicer arientinum* Seeds: **A.** Vigour index; **B.** Seedling growth; **C.**Weight; **a.** Vigour index; **b.** Seedling growth; **c.** Weight (UT1- Red CE; UT2- Yellow CE; UT3- Blue CE; UT4- Navy-Blue CE; UT5- Effluent;T1- Red CE; T2- Yellow CE; T3- Blue CE; T4- Navy-Blue CE; T5- Effluent; C- Control). The values are expressed as mean ± standard deviation (n = 3) and $^{a}p > 0.08$; $^{b}p > 0.06$; $^{*}p < 0.001$; $^{**}p < 0.003$; $^{**}p < 0.02$ compared with the control. (**UT- Untreated, T-Treated**).

Cicer arientinum seedlings grown in distilled water (control) had a shoot length of 6.5 cm, whereas those grown from seeds not treated with the *A. paeoniifolius* crude enzyme were shorter by between 2.5 and 1 cm. The highest reduction at Blue CE dye (UT3) without the *A. paeoniifolius* crude enzyme was found to be 2.5 cm (Table 3 and Figure 3B).

The root length of *Cicer arientinum* seedlings grown in distilled water (control) was 8 cm, whereas the root length of seedlings grown without the *A. paeoniifolius* crude enzyme was reduced between 3.9 and 3.4 cm. All the samples showed a maximum reduction of 3.9 cm except UT5 (untreated effluent), which showed a reduction of 3.4 cm as compared to control (Table 3 and Figure 3B).

The fresh weight of *Cicer arientinum* seedlings in distilled water (control) was 4.96 mg, whereas the fresh weight of seedlings untreated without the *A. paeoniifolius* crude enzyme was reduced by between

0.64 and 1.84 mg. The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 1.84 mg in Red CE (UT1) (Table 3 and Figure 3 C).

Cicer arientinum seedlings grew to 2.87 mg in dry weight when cultivated in distilled water (control), but only 0.34 to 0.72 mg in dry weight when not treated with the crude enzyme from *A. paeoniifolius.* The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 0.72 mg in Blue CE (UT3) (Table 3 and Figure 3 C).

Table 4: Effect of dye and effluent with Crude enzyme from *A. paeoniifolius* on some growth parameters of *Cicer arientinum*

Treatment	**Seed germination Percentage (7th days)**	**Vigour index (Mean±SD)**	**Seedling growth (14th days)**		**Weight**	
			Shoot length (cm.) Avg. (Mean±SD)	**Root length (cm) Avg. (Mean±SD)**	**Fresh weight (mg) (Mean±SD)**	**Dry weight (mg) (Mean±SD)**
T1	80	1064±16.67	6±0.14	7.3±0.38	5.72±0.23	2.89±0.07
T2	70	903±19.87	5.8±0.12	7.1±0.28	5.93±0.09	2.96±0.05
T3	70	959±11.34	6.2±0.21	7.5±0.23	5.39±0.12	3.13±0.09
T4	60	774±9.63	5.7±0.27	7.2±0.36	5.64±0.16	3±0.08
T5	50	645±10.67	5.9±0.11	7±0.29	5±0.11	2.95±0.1
C	100	1240±9.45	5.5±0.18	6.9±0.16	4.96±0.1	2.87±0.12

A study of *Cicer arientinum* seed germination percentages revealed that distilled water seedlings (control) had a 100% germination percentage, while treated seeds with the *A. paeoniifolius* crude enzyme had a germination percentage between 50% and 80% lower than controls. The greatest reduction in germination percentage was reported in the effluent (T5) sample, i.e., 50%, as compared to the control (Table 4).

The vigour index in untreated seedlings (control) of *Cicer arientinum* was 1240, while the vigour index of seedlings treated with the *A. paeoniifolius* crude enzyme was decreased in comparison to control. The maximum reduction was found 645 in effluent (T5) as compared to control (Table 4 and Figure 3a).

Cicer arientinum seedlings grown in distilled water (control) had a shoot length of 5.5 cm, whereas those grown from seeds treated with the *A. paeoniifolius* crude enzyme were increased between 0.2 and 0.7 cm. The highest increase was found in Red CE dye (T1) after treatment with *A. paeoniifolius* crude enzyme, i.e., 0.7 cm (Table 4 and Figure 3b).

The root length of *Cicer arientinum* seedlings grown in water (control) was 6.9 cm, whereas the root length of seedlings grown with the *A. paeoniifolius* crude enzyme was increased between 0.1 and 0.6 cm. The maximum root length increase was found in Blue CE (T3) after treatment with *A. paeoniifolius* crude enzyme (0.6 cm), as reported in Table 4 and Figure 3b.

The fresh weight of *Cicer arientinum* seedlings in distilled water (control) was 4.96 gm, whereas the fresh weight of seedlings treated with the *A. paeoniifolius* crude enzyme was increased between 0.04 and 0.97 mg. The presence of the *A. paeoniifolius* crude enzyme resulted in a maximum increase of 0.97 mg in Yellow CE dye (T2) (Table 4 and Figure 3c).

Cicer arientinum seedlings grew to 2.87 mg in dry weight when cultivated in distilled water (control), but only 0.02 to 0.26 mg in dry weight when not treated with the crude enzyme from *A. paeoniifolius*. The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 0.26 mg in Blue CE dye (T3) (Table 4 and Figure 3C).

All the visual appearances with and without treatment with enzymes in *Cicer arientinum* seedling growth are shown in Figure 4.

The increased number of solids present in the effluent, which causes alterations in the osmotic interaction between the seed and the water, may explain the reason for the lower germination percentage, whereas after seed exposure to crude enzyme from *A. paeoniifolius* in the effluent and dye, the germination percentage in all three plants increased.

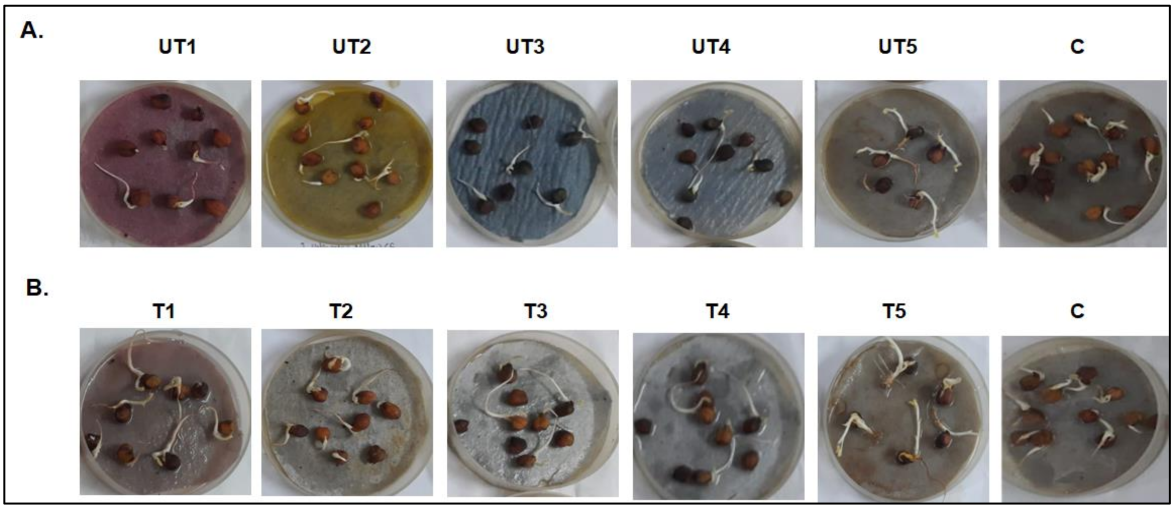

Figure 4: Seedling growth of *Cicer arientinum* **A.** Without crude enzyme from *A. paeoniifolius* **B.** with crude enzyme from *A. paeoniifolius*

The Table 5 and Table 6 compares the germination percentage, vigour index, shoot length, root length, fresh weight, and dry weight of *Triticum aestivum* grown with different samples with distilled water (control), without crude enzyme and with the *A. paeoniifolius* crude enzyme from *A. paeoniifolius.*

Table 5: Effect of dye and effluent without Crude enzyme from *A. paeoniifolius* on some growth parameters of *Triticum aestivum*

Un-treatment	**Seed germination Percentage (7th days)**	**Vigour index (Mean±SD)**	**Seedling growth (14th days)**		**Weight**	
			Shoot length (cm.) Avg. (Mean±SD)	**Root length (cm) Avg. (Mean±SD)**	**Fresh weight (mg) (Mean±SD)**	**Dry weight (mg) (Mean±SD)**
UT1	60	336±6.67	4±0.13	1.6±0.13	2.02±0.11	1.21±0.07
UT2	50	340±8.33	5±0.17	1.8±0.26	1.81±0.09	1.04±0.06
UT3	50	320±10.54	4.5±0.27	1.9±0.15	2.32±0.07	1.05±0.08
UT4	50	130±6.89	1.5± 0.19	1.1±0.07	2.11±0.12	1.63±0.04
UT5	40	208±5.41	3.5±0.15	1.7±0.06	2.61±0.08	1.42±0.12
C	100	800±4.78	6±0.08	2±0.05	2.86±0.1	1.77±0.13

A study of *Triticum aestivum* seed germination percentages reveal that distilled water seedlings (control) had a 100% germination rate, while untreated seeds without the *A. paeoniifolius* crude enzyme have a germination percentage between 40% and 50% lower than controls (Table 5). Lowest seed germination was reported in effluent (UT5) i.e. 40%.

The vigour index of *Triticum aestivum* seedlings grown in distilled water (control) was 800, whereas the vigour index of seedlings grown without the *A. paeoniifolius* crude enzyme was reduced to 130 to 336 (Table 5 and Figure 5 A).

Triticum aestivum seedlings grown in distilled water (control) had a shoot length of 6 cm, whereas those grown from seeds not treated with the *A. paeoniifolius* crude enzyme were shorter between 1 and 4.5 cm. The highest reduction at Navy-Blue CE dye (UT4) without the *A. paeoniifolius* crude enzyme was found to be 4.5 cm (Table 5 and Figure 5 B).

The root length of *Triticum aestivum* seedlings grown in distilled water (control) was 2 cm, while the root lengths of seedlings grown from seeds not treated with the *A. paeoniifolius* crude enzyme were 0.1 to 0.9 cm shorter. Without the *A. paeoniifolius* crude enzyme, the greatest decrease at Navy-Blue CE dye (UT4) was found to be 0.9 cm (Table 5 and Figure 5 B).

The seedlings of *Triticum aestivum* in distilled water (control) had a fresh weight of 2.86 mg, while the seedlings that were not treated with the *A. paeoniifolius* crude enzyme had a fresh weight reduction of 0.25 to 1.05 mg. Yellow CE (UT2) decreased to a maximum of 1.05 mg in the absence of *A. paeoniifolius* crude enzyme. (Table 5 and Figure 5C).

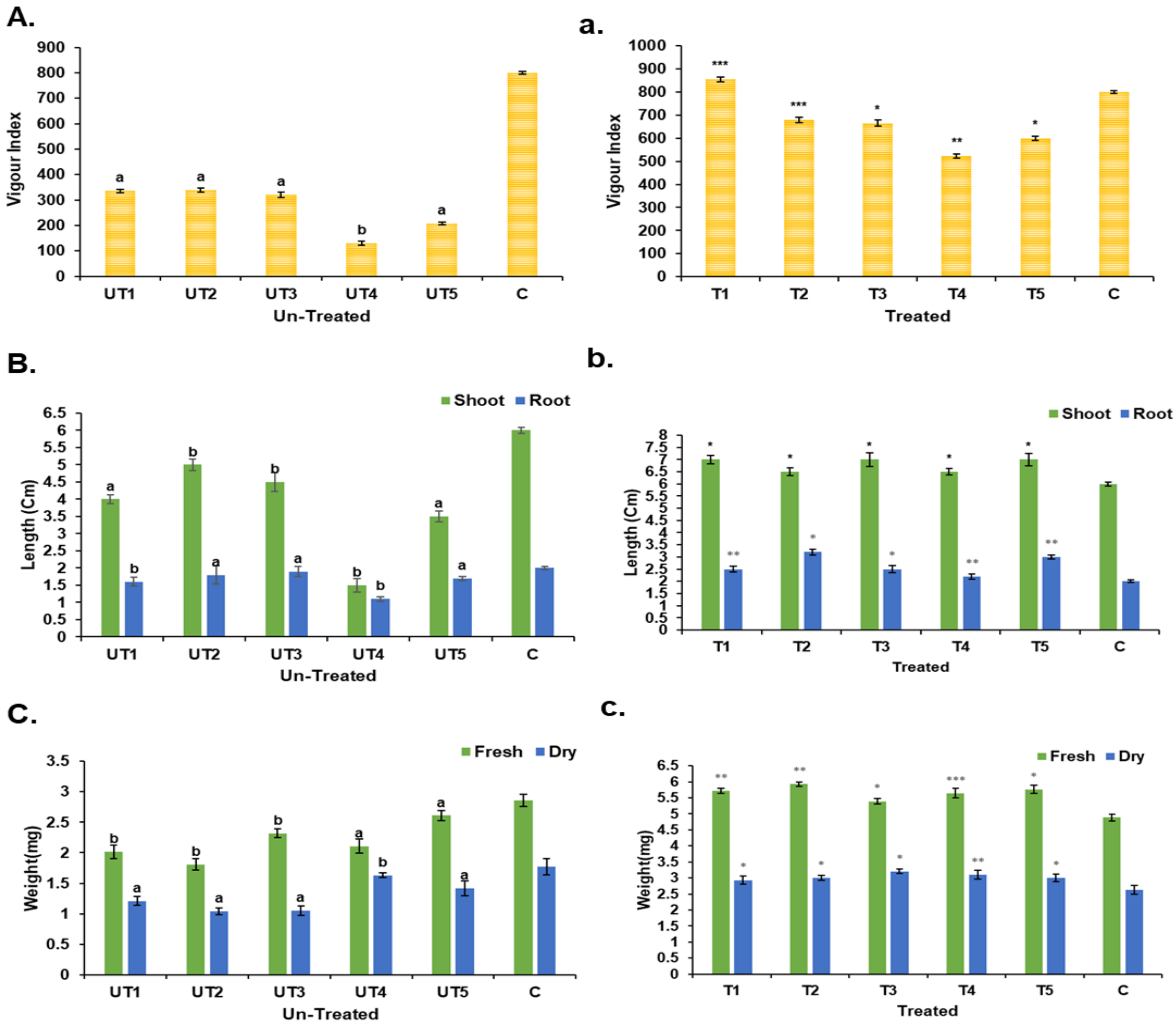

Figure 5- Bar-graph showing the impact of dying and effluent with Crude enzyme from *A. paeoniifolius* on growth parameters of *Triticum aestivum* Seeds: A. Vigour index; B. Seedling growth; C. Weight; a. Vigour index; b. Seedling growth; c. Weight (UT1- Red CE; UT2- Yellow CE; UT3- Blue CE; UT4- Navy-Blue CE; UT5- Effluent; T1- Red CE; T2- Yellow CE; T3- Blue CE; T4- Navy-Blue CE; T5- Effluent; C-Control) The values are expressed as mean ± standard deviation (n = 3) and ap > 0.06; bp > 0.07; *p < 0.001; **p < 0.01; ***p < 0.004 compared with the control.(**UT- Untreated , T-Treated**).

Triticum aestivum seedlings grew to 1.77 mg in dry weight when cultivated in distilled water (control), but only 0.14 to 0.73 mg in dry weight when not treated with the crude enzyme from *A. paeoniifolius*. The absence of the *A. paeoniifolius* crude enzyme resulted in a maximum decrease of 0.73mg in Yellow CE (UT2) (Table 5 and Figure 5 C).

Table 6: Effect of dye and effluent with Crude enzyme from *A. paeoniifolius* on some growth parameters of *Triticum aestivum*

Treatment	Seed germination Percentage (7th days)	Vigour index (Mean±SD)	Seedling growth (14th days)		Weight	
			Shoot length (cm.) Avg. (Mean±SD)	Root length (cm) Avg. (Mean±SD)	Fresh weight (mg) (Mean±SD)	Dry weight (mg) (Mean±SD)
T1	90	855±9.87	7±0.18	2.5±0.13	5.72±0.08	2.93±0.13
T2	70	679±11.65	6. 5±0.16	3.2±0.11	5.93±0.07	3±0.08
T3	70	665±12.67	7±0.28	2.5±0.15	5.39±0.09	3.2±0.07
T4	60	522±8.76	6.5±0.14	2.2±0.1	5.64±0.15	3.1±0.14
T5	60	600±8.98	7±0.25	3±0.09	5.76±0.13	3±0.11
C	100	800±4.78	6±0.08	2±0.05	4.88±0.1	2.63±0.13

A study of *Triticum aestivum* seed germination percentages revealed that distilled water seedlings (control) had a 100% germination percentage, while treated seeds with the *A. paeoniifolius* crude enzyme had a germination percentage between 60% and 90% lower than controls (Table 6). Table 6 displays that the greatest reductions in Navy Blue CE dye (T4) and effluent (T5) concentration were 60% as compared to control, respectively.

The vigour index in untreated seedlings (control) of *Triticum aestivum* was 800, while the vigour index of seedlings treated with the *A. paeoniifolius* crude enzyme was decreased in comparison to the control (Table 6). The maximum reduction was found at 522 in Navy-Blue CE dye (T4) as compared to control (Table 6 and Figure 5a).

Triticum aestivum seedlings grown in distilled water (control) had a shoot length of 6 cm, whereas in the seeds treated with the *A. paeoniifolius* crude enzyme, shoot length increased between 0.5 and 1 cm. The highest increase is reported in Red CE dye (T1), Blue CE dye (T3) and Effluent (T5) after treatment with the *A. paeoniifolius* crude enzyme i.e. 1 cm in all these samples (Table 6 and Figure 5 b).

The root length of *Triticum aestivum* seedlings grown in distilled water (control) was 2 cm, whereas the roots of seedlings grown with the *A. paeoniifolius* crude enzyme were longer between 0.2 and 1.2 cm. The maximum increase in Yellow CE dye (T2) was found to be 1.2 cm. (Table 6 and Figure 5 b).

The *A. paeoniifolius* crude enzyme-treated seedlings showed a rise in fresh weight of between 0.51 and 1.05 mg, while the fresh weight of *Triticum aestivum* seedlings in distilled water (control) was 4.88 gm. Yellow CE dye (T2) increased by a maximum of 1.05 mg when *A. paeoniifolius* crude enzyme was present (Table 6 and Figure 5 c).

When grown in distilled water (control), *Triticum aestivum* seedlings reached 2.63 mg of dry weight; however, when left untreated with the crude enzyme from *A. paeoniifolius*, they only reached 0.3 to 0.57 mg of dry weight. Blue CE dye (T3) decreased by a maximum of 0.57 mg in the absence of *A. paeoniifolius* crude enzyme (Table 6 and Figure 5 C).

Seedling growth images with all the samples of *Triticum aestivum* seedling is shown in Fig. 6.

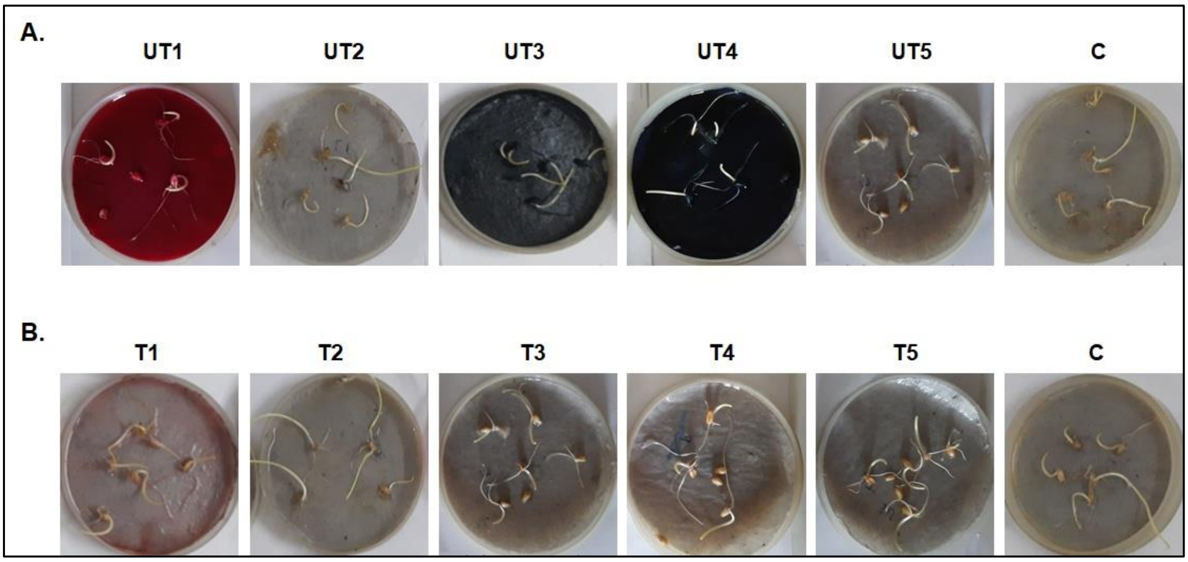

Figure 6: Seedling growth of *Triticum aestivum* **A.** Without crude enzyme from *A. paeoniifolius* **B.** With crude enzyme from *A. paeoniifolius*

Discussions

Phytoremediation is an eco-friendly method of treating wastewater to reduce several types of pollutants that might otherwise be released into the environment. When treated properly, the organic matter and nutrients available in effluents can be utilized by crops, reducing the need for additional fertilizers. The current study found that wastewater from the textile industry reduces seed germination and stunts the development of young plants. Possibly poisonous chemicals and metal ions are present in the corresponding industrial wastewater (Rajasulochana *et al*., 2016; Pandey *et al*., 2020). A detrimental influence on germination is caused by elevated levels of pH, BOD, COD, and other organic loading. Some of the nutrients in the effluent are necessary, but at larger concentrations they become harmful and poisonous to the plants, like *Trifolium pratense, whose* germination is inhibited at 50% effluent concentration (Calheiros *et al*., 2008). *Phaseolus mungo* L. seed germination and seedling growth parameters were used to evaluate the phytotoxicity of the treated and untreated textile industry wastewater. The results demonstrated a considerable decrease in the toxicity of the treated textile industry wastewater (Kishor *et al*., 2021). After post-treatment textile wastewater, the phytotoxicity assessment on *Sorghum bicolour* and *Triticum aestivum* revealed 80% and 60% reduction, respectively (Ayed *et al*., 2020). Seedling growth and development are stimulated when exposed to a mixture of effluent and dye containing crude enzyme from *A. paeoniifolius*. Maximum 90 % germination was reported in various dyes and effluents in *Vigna radiata* and *Triticum aestivum* after treatment with *A. paeoniifolius,* i.e., T5 and T3 in *Vigna ratiata* and T1 in *Triticum aestivum,* whereas *Cicer arientinum* shows maximum 80% germination, i.e., reported in T1 dye. The larger amount of particle concentration in the effluent and dye may be responsible for the change in osmotic interaction between the seed and water, resulting in a lower seed germination percentage without exposure to crude enzymes from *A. paeoniifolius*. Crude enzyme from *A. paeoniifolius* positively impacts seed germination% of *Vigna radiata, Cicer arientinum*, and *Triticum aestivum,* whereas without treatment it had no significant effect. Plant growth parameters after treatment with crude enzyme from *A. paeoniifolius were* observed to boost seedling development. The exposure of seedlings to various dyes and effluent after treatment with enzyme shows improved growth and overall seedling development.

Conclusion

Overflowing effluents have a negative impact on seedling germination and growth. However, treated effluent and dyes had no detrimental effects on germination%, vigour index, root, and shoot length. According to the current study's findings, the growth features of *Vigna radiata*, *Cicer arientinum*, and *Triticum aestivum* have been significantly impacted by effluent waste and dyes that have been treated with *A. paeoniifolius* crude enzyme. Among all three seed plants, *Vigna radiata* shows the highest seed germination percentage, Vigour index, shoot length, and root length.

Cultivations of *Vigna radiata*, *Cicer arientinum*, and *Triticum aestivum* may benefit from irrigation with treated dye and effluent exposed to crude enzyme from *A. paeoniifolius*. However, it is important to note that wastewater irrigation should be implemented with appropriate safeguards and proper monitoring needed to ensure the safety of both crops and consumers. It is recommended to conduct soil and water quality assessments, establish threshold levels for contaminants, and adopt best management practices for effluent use.

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Conflict of Interest

The authors declare that is there is no conflict of interest.

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