Bioremediation of Textile and Tannery Wastewater Using *Chlorella Vulgaris* and Impact of *Chlorella* Treated Textile and Tannery Wastewater on Seedling Growth of *Vigna radiata* and *Sesamum indicum L.*

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***Abstract*:** A number of micro-algal species have been used in the treatment of industrial wastewater. The treated water from these treatments was perfectly reused for industrial purposes. But, the present study precedes *Chlorella vulgaris* for wastewater (textile and tannery) treatment. After treatment, the water was shower to seeds (*Vigna radiata* and *Sesamum indicum L.*) to monitor its toxicity. Due to the high algal count and considerable level of physiochemical parameters (EC, pH, BOD, COD, TS, TDS, TH and chloride), the seed germination and plant growth was relatively equal in tap water, algal grown tap water, algal treated textile and tannery wastewater.

Keywords: Textile wastewater, Tannery wastewater, *Chlorella vulgaris*, *Vigna radiata* and *Sesamum indicum L.*

I. INTRODUCTION

The 20th century not only increases population and also rapidly increases the industries to satisfy the human needs. Among the industries, textile and tannery were found to discharge large amount of wastewater onto the river basins. There are about 3000 tanneries and 21076 textile units in India (Sujatha and Gupta 1996, Bal 1999). FAO/WHO in 1993 stated that high metal concentration from leather effluents when deposited in river basins (Buriganga) creates severe health problems like cancer, organ damage, nervous system damage and in extreme causes death (FAO/WHO 1993). The colloidal substances from textile effluents along with the color and oily scum prevent the penetration of sunlight necessary for photosynthesis (HSRCS 2005). Phycoremediation is the only salvation to eradicate the pollution created by textile and tannery wastewater to mankind.

It is universally acknowledged that algae are the only source for the natural purification of industrial wastewater (Han et al 2000). The removal or biotransformation of pollutants including xenobiotics from wastewater by using micro or macro algae is known as phycoremediation (Olguín 2003). Phycoremediation is low-cost and effective due to high intake of inorganic nutrients from wastewater (Bolan et al 2004).

As a major contributor in the fertility of soil, algae are used for plant growth. It has been proved that the soaked seeds of *Medicago sativa L* with algae (*Spirogyra sp* and *Oscillatoria sp*) showed faster germination than in tap water (Brahmbhatt and Haresh 2015). As the chemical fertilizers are available with drawbacks, 15 million metric tons of algal products were used as bio-fertilizer, bio-stimulant and as nutrient supplements annually (Sharitamadari et al 2011).

Numberless research have proved that algae serves as a best source for the growth of plant, but the current work aims on treating industrial (tannery and textile) wastewater with *Chlorella vulgaris* and monitoring the growth of *Vigna radiata* and *Sesamum indicum L.* on tap water, algal grown tap water, algal treated wastewater.

II. MATERIALS AND METHODS

2.1 Collection of industrial wastewater and analysis of physio-chemical parameters

Both the textile and tannery wastewater were collected from different sites. The tannery wastewater was collected from the leather tannery outlet located in Erode district, Tamilnadu. The textile wastewater was collected from the dyeing unit of textile outlet located in Tirupur district, Tamilnadu. The samples were stored at 4ºC in dark for further deactivation. The physio-chemical parameters such as pH, Electrical Conductivity, Biological Oxygen Demand, Chemical Oxygen Demand, Total Solids, Total Dissolved Solids, Total Hardness and Chloride were analysed for textile, tannery wastewater using standard methods of before and after treatment (Clesceri et al 1989).

2.2 Collection of algal strain and seeds

The microalgal strain *Chlorella vulgaris* DPSF01 was collected from Bharathidasan Univesity, Department of Marine Science. The culture was inoculated in BBM (Bold Basal Medium) and maintained at 20ºC–23ºC under fluorescent light of 12 hr and

remaining 12 hr in dark (Nichols 1973). Seeds of *Vigna radiata* and *Sesamum indicum L* were collected from Tamilnadu Agricultural University, Coimbatore. Seeds were stored on aluminium foil bags at 4ºC until use.

2.3 Cultivation of algae on textile and tannery wastewater

The alga was inoculated separately in textile and tannery wastewater. The different dilutions are 10%, 20% and 30% of wastewater with tap water containing initial algal count of 5×10^3 cells/mL. This initial count of alga was also grown in normal tap water without any wastewater. Tap water without algae was kept as a control. The tap water and wastewater with algal cells are maintained at 20±2ºC in photoperiod of 12hr light and 12 hr dark. The experiments were carried out for 28 days (Ajayan et al 2015).

2.4 Estimation of algal growth

The cell count was estimated at different intervals from 7th day, 14th day, 21st day and 28th day. 10µl of the algal cells from different dilutions (10%, 20% and 30%) and tap water were taken and loaded in hemocytometer slides. Number of cells was counted and estimated for ml according to Lackey Drop Micro Transect Counting Method (Lenore 1998).

2.5 Chlorophyll estimation

Chlorophyll *a* and *b* was estimated for both textile and tannery wastewater dilutions and also in algal grown tap water. 20mL of culture was centrifuged at 10,000 rpm for 10 min. The pellet was mixed with DMSO and again the mixture was centrifuged at 5000 rpm for 10 min for re-extraction. After centrifugation, the supernatant was measured at 645nm and 663 nm absorbance in spectrophotometer according to Arnon, 1949.

2.6 Seed germination by plate method

Seeds of *Vigna radiata* and *Sesamum indicum L* were air-dried and soaked in 10% dilution of tannery and textile wastewater and as well as in algal grown tap water for 24 hrs. The seeds were grown only in 10% dilution of both wastewater and algal grown tap water is because of high algal cell count. After soaking, the seeds were allowed to germinate in a Petri-dish containing 5ml of algal extract. Seeds without algal extract serve as a control (plate-1). Plate-2 contains pure algal extract from tap water; plate-3 contains algal extract from textile wastewater and plate-4 with algal extract from tannery wastewater. The Petri-dishes were placed under illumination at 20ºC for 5 days (Sedigheh 2010).

2.7 Plant growth by pot method

The pot experiment was conducted in 1 liter pot for 10 days. Ten healthy seeds (*Sesamum indicum L* and *Vigna radiata*) were selected and sown on the pot filled with equal amount of (1:1:1) clay, soil and sand. The seeds were sown under 1 cm depth. The seeds were sprayed with 200 ml of algal extract except the seeds grown on tap water which serve as a control (pot-1). The seeds of pot-2 were sprayed with pure algal extract from tap water, pot-3 with algal extract from textile wastewater and pot-4 with algal extract from tannery wastewater. Percentage of seed germination, shoot length and root length of 10 days old plant from each pot were measured (Sivasankari 2006).

2.8 Statistical analysis

Experiments were carried out with triplicates. Results were presented with standard error of the mean.

III. RESULTS AND DISCUSSION

3.1 Estimation of cell count

The count of green algae reached high at the end of the treatment in 10% dilution. The growth was enumerated once in 7 days interval for all the dilutions. Limited growth was observed in 20% and 30% dilutions. In textile wastewater, the maximum cell count is 1272×10^3 cells/mL in 10% dilution (Figure 1a), whereas in tannery wastewater it is 1124×10^3 cells/mL (Figure 1b). The cell count in 10% dilution were more or less equal to the tap water (1310×10^3 cells/mL) (Kheiralla et al 2014).

3.2 Analysis of chlorophyll count on textile and tannery wastewater before and after treatment

Figure 2a and b indicate the chlorophyll count on different concentration of textile wastewater and figure 3a and b indicates the count on tannery wastewater. Chlorophyll *a* (4.77 µg/ml and 3.95 µg/ml) and *b* (2.42 µg/ml and 1.53 µg/ml) were found to be high on 10% dilution of both textile and tannery wastewater. 30% showed low count of chlorophyll *a* (2.66 µg/ml and 1.99 µg/ml) and *b* (0.86 µg/ml and 0.56 µg/ml) on textile and tannery wastewater. This is because the nutrients present in tap water increases the growth of algae whereas the heavy metals in textile and tannery wastewater suppress the growth (Hagemeyer 1999).

3.3 Physio-chemical properties of textile and tannery wastewater before and after phycoremediation

During the phycoremediation, the colour of both textile and tannery wastewater changed to pleasant green colour due to rapid growth of algae. Table 1 and 2 shows the variation of physio-chemical parameters in textile and tannery wastewater before and after treatment. BOD in textile and tannery wastewater was reduced from 513 mg/l to 101 mg/l; 1356 mg/l to 257 mg/l and COD was (1332 mg/l to 289 mg/l; 2413 mg/l to 1058 mg/l) respectively. The photosynthetic activity of microalga reduced BOD and COD to about 50% after 28 days of treatment (Colak and Kaya 1988). After treatment, the pH of textile and tannery wastewater was changed from acidic to base. By the process of photosynthesis, the microalga reduces dissolved CO_2 which eventually raises pH level to base (Borowitzka 1998). The nutrient uptake from textile and tannery wastewater by *C. vulgaris* decreases total solids (4969 mg/l to 1097 mg/l; 6556 mg/l to 2036 mg/l) and total dissolved solids (4122 mg/l to 976 mg/l; 5446 mg/l to 1396 mg/l) to considerable level (Hanumantha et al 2011). After treatment, total hardness in textile (1034 mg/l to 440 mg/l) and tannery (876 mg/l to 312 mg/l) wastewater was reduced above 40%. In case of *Chlorococcum humicola*, the reduction of total hardness from industrial effluents was only 25% in lab scale (Sivasubramanian et al 2012).

Table 1. Physio-chemical parameters of tannery wastewater before and after treatment

S.NO	Physio-chemical parameters	Tannery wastewater	Algal treated wastewater
1	pH	5.10±0.26	7.79±0.31
2	EC (dsm^{-1})	10.01±0.20	2.69±0.41
3	Biological Oxygen Demand (mg L^{-1})	1356±7.93	257±6.24
4	Chemical Oxygen Demand (mg L^{-1})	2413±6.11	1058±8.50
5	Total Solids (mg L^{-1})	6556±7.37	2036±7.76
6	Total Dissolved Solids (mg L^{-1})	5446±7.09	1396±7.54
7	Chloride (mg L^{-1})	385±4.04	157±5.56
8	Total Hardness (mg L^{-1})	876±4.35	312±4.72

Note: The values are represented with standard Error of the mean of triplicates ($p < 0.05$).

Table 2. Physio-chemical parameters of textile wastewater before and after treatment

S.NO	Physio-chemical parameters	Textile wastewater	Algal treated wastewater
1	pH	6.02±0.10	8.52±0.08
2	EC (dsm^{-1})	9.06±0.13	2.66±0.18
3	Biological Oxygen Demand (mg L^{-1})	513±6.24	101±8.14
4	Chemical Oxygen Demand (mg L^{-1})	1332±6.11	289±6.65
5	Total Solids (mg L^{-1})	4969±4.16	1097±7.02
6	Total Dissolved Solids (mg L^{-1})	4122±5.68	976±7.37
7	Chloride (mg L^{-1})	259±4.72	116±6.08
8	Total Hardness (mg L^{-1})	1034±5.13	440±5.29

Note: The values are represented with standard Error of the mean of triplicates ($p < 0.05$).

3.4 Percentage of seed germination by plate method (Table 3)

The seeds of *Sesamum indicum L* and *Vigna radiata* soaked with pure algal extract from tap water started germination after 36 hours and the germination percentage was also high (80% & 68%).The faster and high germination percentage is because of algae as nitrogenous nutrient to the seeds (Nanda et al 1991). Lower seedling growth (4.60 cm and 3.25 cm) was observed with seeds soaked in algal extract from tannery wastewater. The high salt content of tannery wastewater decreases the uptake of water for the seedling growth (Ajmal and Khan 1983) (Figure 4 a and b).

Table 3. Effect of algal extract on seedling growth of by plate method

	Vigna radiata			
	Control	**Plate-1**	**Plate-2**	**Plate-3**
% Germination	70±1.15	80±0.57	50±1.73	30±1.52
Length of the radicle	10.36±0.30	12.55±1.01	5.22±2.43	4.60±1.36
	Sesamum indicum L			
% Germination	62±1.73	68±2.08	41±2.30	35±3.21
Length of the radicle	7.22±0.77	8.55±0.82	4.12±0.29	3.25±0.11

Control – Seeds soaked on tap water.

Plate-1 – Seeds soaked on algal grown tap water.

Plate-2 – Seeds soaked on algal treated textile wastewater.

Plate-3 – Seeds soaked on algal treated tannery wastewater.

3.5 Estimation of plant growth parameters by pot method (Table 4)

The initial part of this research revealed high algal cell count in normal tap water whereas lower cell count in tannery wastewater. Therefore greater plant growth (shoot length = 8.58 cm and 6.32 cm; root length = 6.86 cm and 4.00 cm) was seen in pot sprayed with algal extract from tap water and less plant growth (shoot length = 3.45 cm and 3.44 cm; root length = 2.95 cm and 1.05 cm) in pot sprayed with algal extract from tannery wastewater. These results confirms the findings of Venkataraman and Neelakantan in 1967 reported that growth substances (Auxins, Amino acids and Sugars) and vitamins (Vitamin-B_{12}, Folic acid, Nicotinic acid and Pantothenic acid) produced by algae enhances high plant growth (Figure 5 a and b).

Table 4. Effect of algal extract on plant growth of by pot method

	Vigna radiata			
	Control	**Pot-1**	**Pot-2**	**Pot-3**
% Germination	82±2.08	93±2.30	64±2.64	41±1.73
Shoot length (cm)	7.14±0.63	8.58±0.27	5.24±0.04	3.45±0.08
Root length (cm)	5.69±0.17	6.86±0.25	3.57±0.12	2.95±0.03
	Sesamum indicum L			
% Germination	73±4.93	81±5.85	56±3.05	40±3.21
Shoot length (cm)	5.22±0.04	6.32±0.08	4.10±0.14	3.44±0.07
Root length (cm)	3.12±0.10	4.00±0.01	2.00±0.05	1.05±0.02

Control – Seeds poured on tap water.

Plate-1 – Seeds poured on algal grown tap water.

Plate-2 – Seeds poured on algal treated textile wastewater.

Plate-3 – Seeds poured on algal treated tannery wastewater.

IV. CONCLUSION

Inoculating the initial count of algae (5×10^3 cells/ml) in normal tap water and diluted wastewater have enhanced the growth of *Sesamum indicum L* and *Vigna radiata* to neutral level. Thus, by increasing the initial count of algae on tap water and wastewater, preferable purification of wastewater and thereby parallel increase in plant growth can be achieved.

V. ACKNOWLEDGEMENTS

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VI. CONFLICT OF INTEREST

Conflict of interest declared none.

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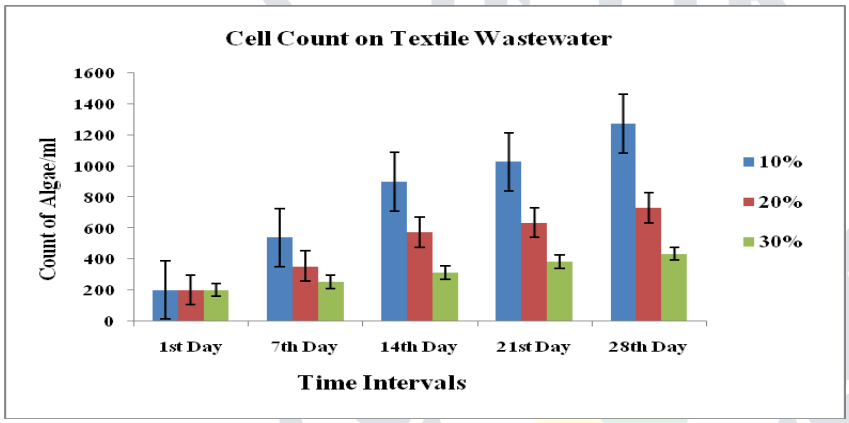


Figure 1a. Cell count on textile wastewater

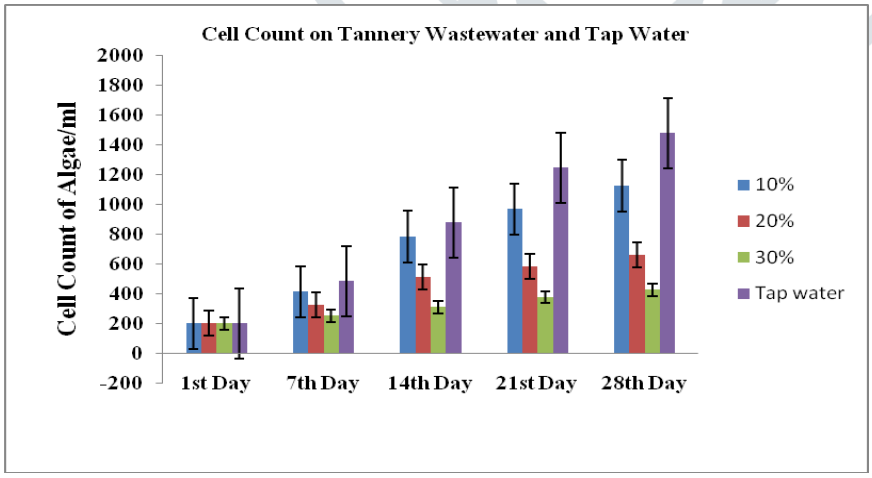


Figure 1b. Cell count on tannery wastewater and tap water

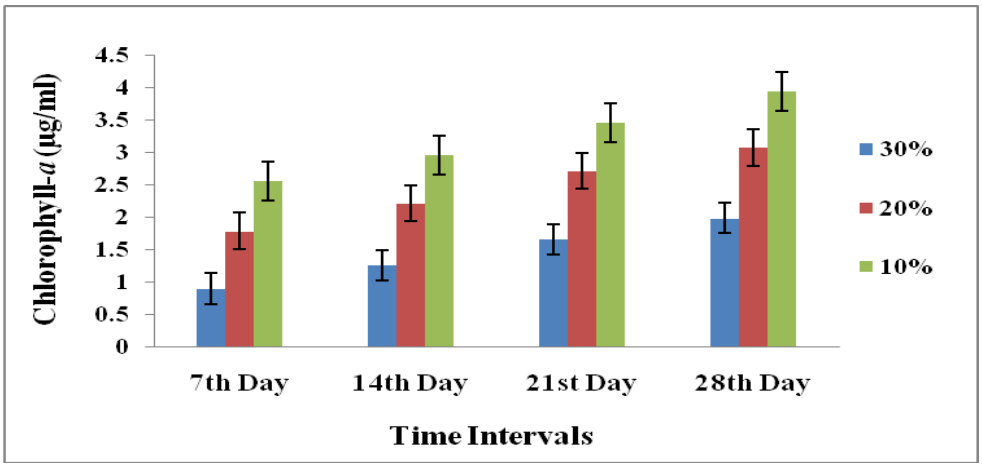


Figure 2a. Estimation of chlorophyll-*a* on textile wastewater

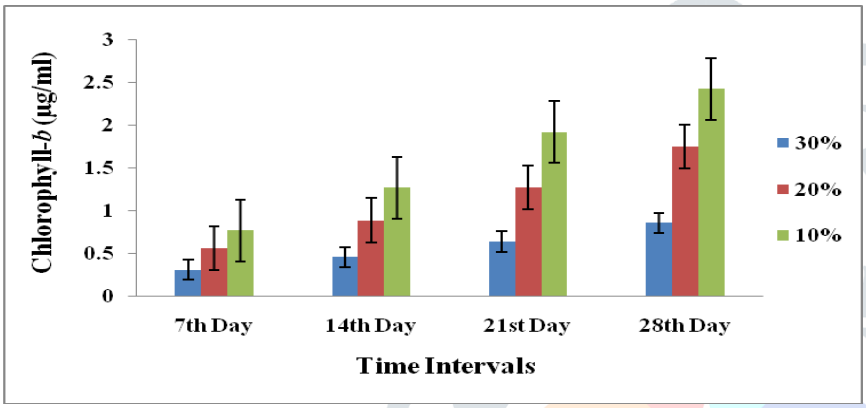


Figure 2b. Estimation of chlorophyll-*b* on textile wastewater

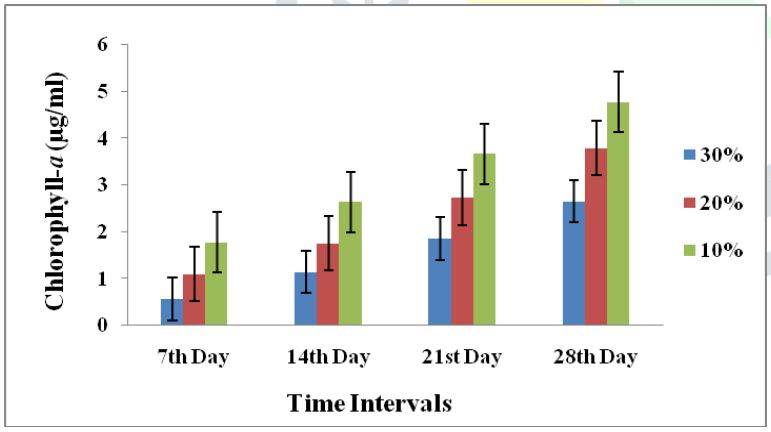


Figure 3a. Estimation of chlorophyll-*a* on tannery wastewater

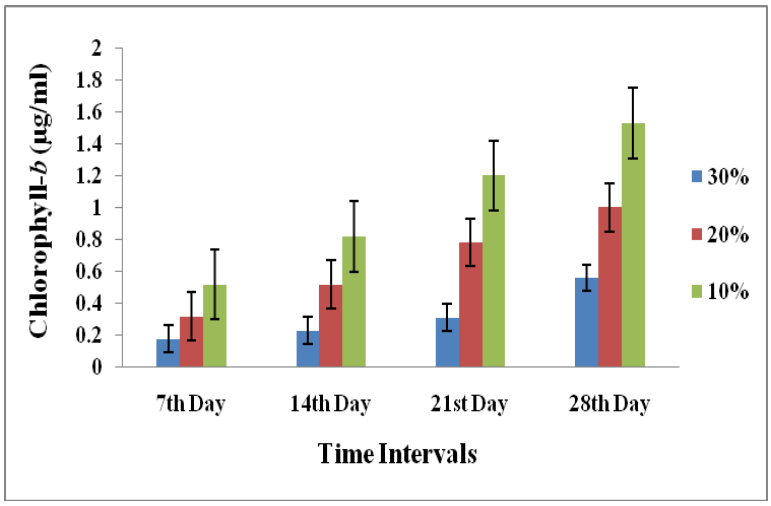


Figure 3b. Estimation of chlorophyll-*b* on tannery wastewater

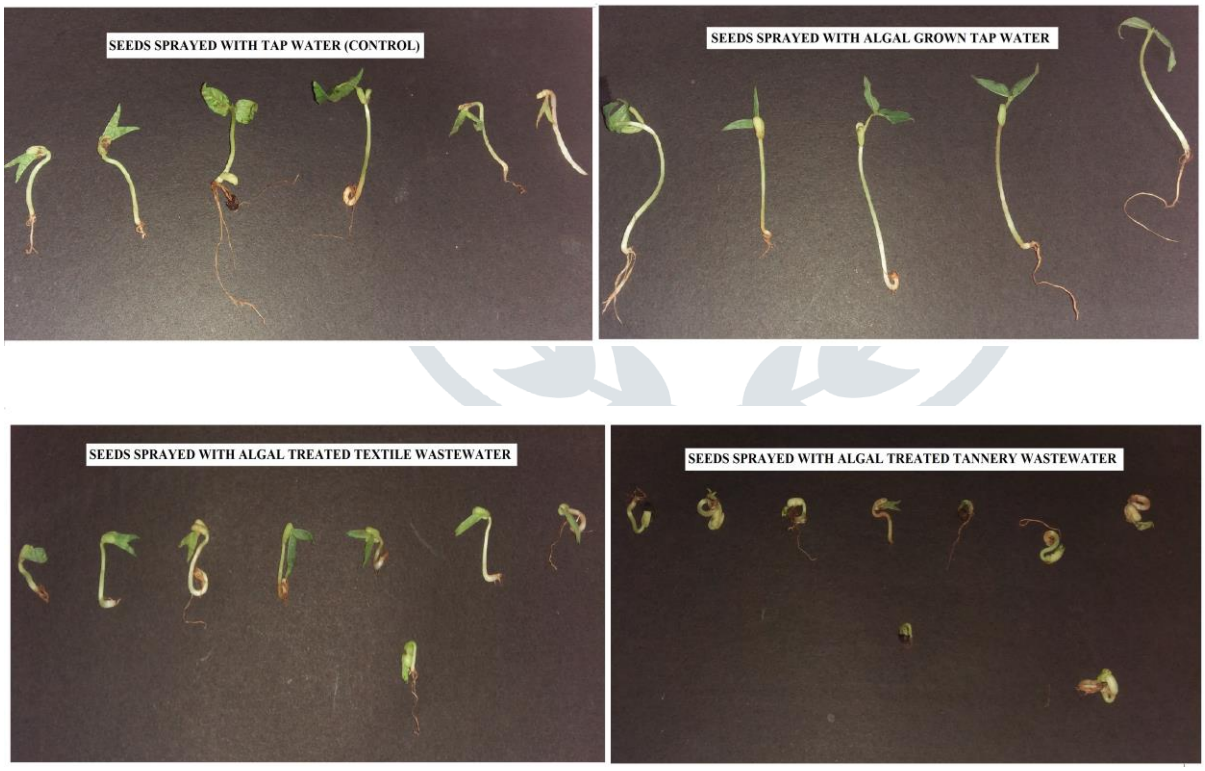


Figure 4a. *Vigna radiata* grown by plate method

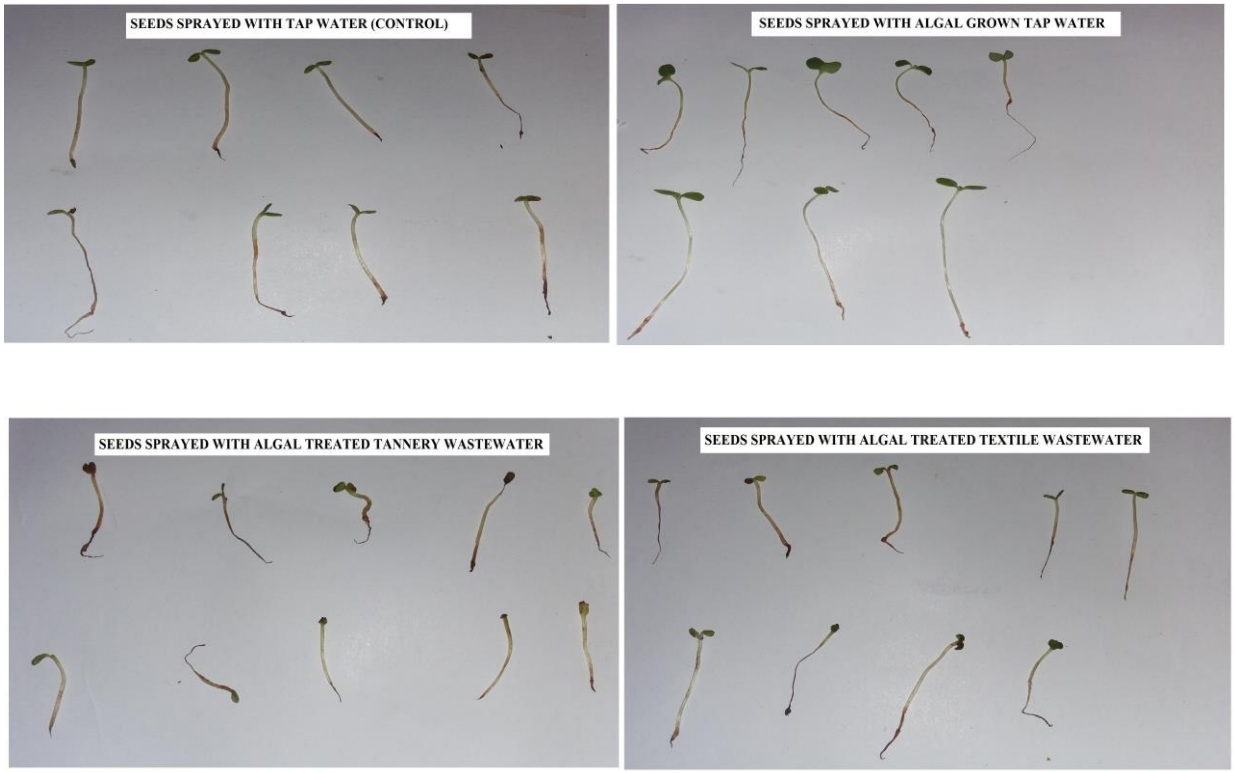
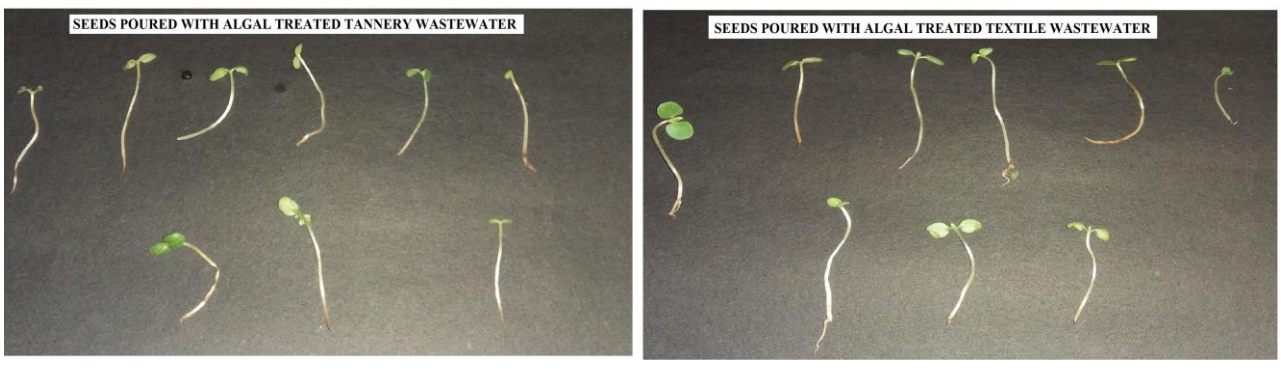


Figure 4b. ***Sesamum indicum L*** **grown by plate method**

Figure 5a. ***Vigna radiata*** **grown by pot method**



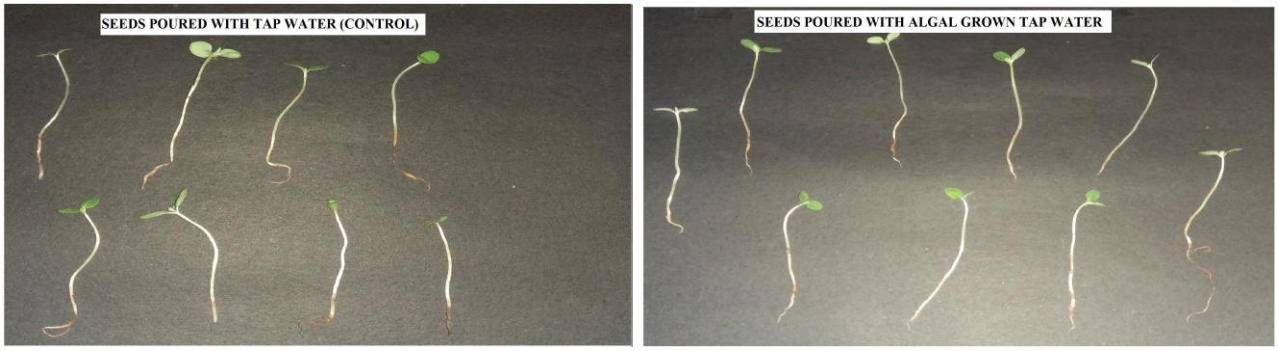


Figure 5b. ***Sesamum indicum L*** **grown by pot method**