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Biological treatment of textile wastewater and its re-use in irrigation: Encouraging water efficiency and sustainable development

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Abstract: The present study focused on the isolation of potential bacteria from contaminated soil of textile industries and subsequent employment of those organisms in treatment of textile waste-water. Wastewater was treated by novel isolates and the biologically treated wastewater was used for the irrigation (phytotoxicity evaluation) of two important edible crop plants (*Brassica nigra* and *Cyamopsis tetragonolobus*). For this, plants were grouped as I, II, III and IV that received the tap water, raw effluent, chemically treated and biologically treated wastewater respectively. 46 bacterial isolates were obtained and optimization of parameters revealed that one strain, namely UBL-27 (*Comamonas sp. UBL 27)* decolorized the wastewater to a max. of 80% in static (anoxic) condition at pH 8 in 24 hours at 32^{o}C. There was a remarkable performance in the germination percentage under biologically-treated wastewater to about 83.6% when compared to that of Control Group producing 92.9%. In contrast to this, the germination % was significantly too low ($p \leq 0.05$) in the other cases with the raw wastewater and chemically treated wastewater. The wastewater had marked effect on the growth of the *Brassica nigra*, the height of the plant was higher in the biologically treated effluent (11.2 ± 0.4 cm) and control group (12.1±0.2) than Group II (8.9±.17 cm) and Group III (9±0.2 cm). Weight of the plant was 1.95±0.35 g and 1.68±0.47 g in Group I and Group IV. It was significantly lower in case of Group II and Group III. In *Cyamopsis tetragonolobus*, heights of the plant among the four groups at the end of 80 days were 102.3±3.4, 52±7.6, 45.3±4.9 and 92.8±5 cm respectively. Similarly, no. of leaves/plant among the four groups was 49.2±3.2, 26.8±4.5, 32±2.4 and 47±4.5. Total yield of the plant under the experimental area for Group I was 3.15±0.09 kg while that of the Group IV was 2.92±0.09 kg. The yield was significantly lower in the Group II and III such as 1.67±0.17 kg and 2.06±0.22 kg respectively. To consolidate, the raw effluent has decreased the yield by more than 45% ($p \leq 0.05$) while that of the chemically treated group by more than 30%. Though, biologically treated wastewater may not be absolutely fit for drinking purposes or for recycling in dyeing processes, it is proved from this, that the eco-friendly alternative can be used for the irrigation purposes beside abatement of water and soil pollution.

Keywords: Textile Wastewater, Biodegradation, Comamonas Sp., Water Efficiency, Phytotoxicity, Textile Wastewater.

1. Introduction

India faces the serious problem of natural resource scarcity, especially that of water in view of population growth and economic development. Groundwater is the major and also an important source of water for the agricultural and industrial sectors in India. During the post-liberalization period of the Indian economy, the textile industries grew rapidly due to the availability of cheap water and labor. The cheap water that is rapidly pumped from underground aquifers is a major factor in the success of textile industries in contributing to India's economic growth. For instance, the textile valley of India, 'Tirupur-city' in the southern state of Tamil Nadu, is one of the largest and fastest growing urban agglomerations, also the textile hub of India and a vast generator of employment, income and foreign exchange - is gaining universal recognition as the leading source of hosiery, knitted garments, casual wear and sportswear.

Despite the recent rapid growth in industrial production, agriculture is still an integral part of India's economy and society. There is a constant competition over water between farming families and industrialists seeking to commodify the resource base for commercial gain. According to the Ministry of Water Resources, industrial water use in India stands at about nearly 6 per cent of total freshwater abstraction and the demand is expected to increase dramatically. On the other hand, Indian agriculture sector claims 90% of total water resources and has caused groundwater depletion. Analyses warn that the water demand in India will overshoot the current supply in the near future. Another major source of India's water supply is surface water, which is highly monsoon dependent. Only 48% of rainfall ends up in Indian rivers, especially in the peninsular rivers like 'Cauvery' in South India. Unfortunately, due to lack of storage and crumbling infrastructure, only 18% of the water can be utilized making water scarcity a critical problem (UNICEF, 2002). Climate change also has an effect on rainfall pattern and is exacerbating the depleting supply of water.

Invariably, ground water and surface water pollution due to unfettered economic growth and poor wastewater management practices of textile industries further worsen the issue of water scarcity. This acute water scarcity crisis in India along with pollution gives reason for concern and the need for appropriate water management practices focusing attention on water conservation, efficiency in water use, wastewater treatment and its recycling. Regardless of the many benefits from a thriving economy, considering both volumes discharged and wastewater composition, the wastewater generated by the textile industry is rated as the most polluting among all industrial sectors (Asgher et al, 2009; Lopez et al, 2006; Vanndevivera et al, 1998).Growing awareness on the impact of pollution in groundwater and surface water, textile wastewater treatment is now receiving greater attention. During the last few years, new and tighter regulations coupled with increased enforcement concerning wastewater discharges have been established in many countries including India. This new legislation, in conjunction with international trade pressures such as increasing competition and the introduction of 'ecolabels' for textile products on the European and US markets, is threatening the very survival of the Indian textile industry.

Considering the vast potential of textile industries in contributing to the Indian economy and supporting millions of people for employment, it becomes mandatory to save the industries from shutting down due to constraints of the Pollution Control Board of the country. At the same time, environmental deterioration also needs immediate attention for preventing any further damage and also to find a viable alternative to the problem. Though physico-chemical methods for wastewater treatment have been accepted and followed by textile industries, they are not cost-effective and involve large volumes of sludge disposal. Accumulation of such concentrated sludge poses a lot of practical difficulties in thedisposal of wastewater. Presently, sludge is being deposited into the lands owned by the textile industries converting them into waste lands (Anjaneyulu et al, 2005; Robinson et al, 2001).

On the other hand, biological treatment could achieve greater efficiencies in the decolorization and detoxification of textile wastewater by making use of the native microorganisms. These microorganisms that are present in the soil of the textile industrial areas, where the dumped dye wastewater contains a lot of synthetic compounds, adapt themselves to the adversity of their micro-environment over the ages due to their persistency. There are a lot of reports suggesting the use of native bacterial isolates that have the capacity to decolorize the dyes that are commonly used in the respective textile industries (Asad et al, 2007; Ali, 2010; Jadhav et al, 2010; Saratale et al, 2010).Since many synthetic dyes and their derivatives are carcinogenic and mutagenic, the process of bioremediation requires not only the decolorization of the water containing the dye but also the complete degradation or detoxification of it (Couto, 2009; Forss and Welander, 2009; Kaushik and Malik, 2009). The re-use of bioremediated textile wastewater for agricultural irrigation is often viewed as a positive means of recycling water due to the potential large volumes of water that can be used.

The present study was an attempt in finding an economically and environmentally (eco) friendly solution for the prevailing textile wastewater pollution. It is focused on the isolation of potential microorganisms (especially bacteria) from the contaminated soils of the industrial areas of Tirupur district, Tamil Nadu, South India, and the subsequent employment of those organisms in decolorization, degradation and detoxification of textile wastewater, while re-using the water for irrigation thereby reducing the demand–supply gap on water, encouraging water efficiency and sustainable development. The remainder of this paper is organized into three sections: section one describes the methodology adopted for the biological treatment of the textile wastewater and phytotoxicity studies, section two presents the results and discussion and the last section provides conclusions and policy implications.

2. Materials and Methods

Textile Wastewater Collection: The raw discharge (Raw wastewater/ wastewater) of the dyeing unit of United Bleacher's (UBL) Pvt. Ltd, Mettuppalayam, Tamil Nadu, India, was collected in barrels and transported to the laboratory within 24 hours.

Isolation and screening of textile waste water decolorizing microorganisms: Soil samples were collected from five different sites around the industry including the drains. All the soil samples were mixed and used for the isolation of dye-decolorizing microorganisms due to the decades-long usage of the location since the establishment of the industry. The soil samples were serially diluted by following the standard protocol and the dilution series from 10^{-2} to 10^{-7} was plated in Nutrient Agar medium (Elisangela et al, 2009). Each dilution was maintained in triplicates. All the plates were incubated at 37°C for 24 h.Pure cultures were raised and maintained on nutrient agar slants at 4°C.

*Decolorization experiments:*A loopful of each isolated bacterial culture was inoculated into a separate 250 ml Erlenmeyer flask containing the raw wastewater in nutrient broth and incubated for 24 h at 37°C for the initial screening of the ability of the isolates to decolorize the wastewater. Aliquots of the culture (3 ml) were withdrawn at different time intervals and centrifuged at 5000 rpm for 15 min to separate the bacterial cell mass. Decolorization was determined by measuring the absorbance of the decolorization medium at absorption maxima, and the percentage of decolorization was calculated as follows: (%) Decolorization = {(Initial absorbance – Observed absorbance) / Initial absorbance} X 100. All decolorization experiments were performed in triplicates (Elisangela,et al, 2009; Saratale,et al, 2006).

*Growth medium:*The growth medium used for this study was nutrient broth. However, to evaluate the nutritional requirements and to optimize the decolorization process of the isolate, the experiments were conducted with minimal media (mg L^{-1}) containing Glucose 1800; $MgSO_4.7H_2O$ 250; KH_2PO_4 2,310; K_2HPO_4 5,550; $(NH_4)_2SO_4$ 1,980. All chemicals used were of highest purity available and of analytical grade.

*16s rDNA sequencing:*The chromosomal DNA of the strains with the best decolorization potential was isolated according to the procedure described earlier (Rainey,1996). A partial DNA sequence for 16S rRNA gene was amplified by using 5' - ATG GAT CCG GGG GTT TGA TCC TGG CTC AGG-3' (forward primer) and 5'-TAT CTG CAG TGG TGT GAC GGG GGG TGG-3' (reverse primer) (Jing et al, 2004; Senthil Kumar et al, 2010). The nucleotide sequence analysis of the sequence was done at Blast-n site at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST).*Phytotoxicity studies:*To assess the toxicity of the above biological treatment and to compare it with that of the chemically treated wastewater (physico-chemical treatment followed in the industry) and untreated (raw effluent) wastewater, black mustard (*Brassica nigra* L.) and cluster beans (*Cyamopsis tetragonolobus* L.) were chosen.

*Germination index and morphological studies:*Emergence of shoot to 0.5cm in length or more was considered germination of the seeds and the germination percentage was determined (Kaushik, 2005). *Cyamopsis tetragonolobus L.* (cluster beans) was chosen for the field trial in which the plants were grouped as I, II, III and IV receiving tap water, raw wastewater (untreated), chemically treated wastewater and biologically treated wastewater, respectively (10 x 15 square feet area each). The morphometric parameters (shoot and root length; dry and wet weight of the whole plant, root and shoot; no. of leaves, nodes, pods, seeds per pod and pod length); yield parameters (weight of a pod, total yield of a plant); and biochemical parameters (chlorophyll, total carbohydrate, protein and reducing sugar) were compared among the treatment groups.

*Statistical analysis:*The data recorded in all the experiments were subjected to Two-Way Analysis of Variance (ANOVA) using MS-Excel (Office 2007). Readings were considered significant when P was $p \leq 0.05$.

3. Results and Discussion

From the soil sample taken at the United Bleacher's Pvt. Limited, Mettuppalayam, Tamil Nadu, bacterial cultures were raised in the laboratory and 46 bacterial isolates were identified based on their distinct colony morphology and was named from UBL 01 to UBL 46. Among the 46 bacterial isolates tested for decolorizing the wastewater in the nutrient medium, only six bacterial cultures demonstrated promising decolorizing activity with over 45% on an average, in shaking condition within 72 h when optically measured at 598nm (data not shown). Those six isolates namely UBL02, 03, 04, 23, 24 and 27, were subjected to 16S rDNA sequencing method of identification and found to be *Enterococcus faecalis* (HM451428), *Bacillus thurungiensis* (HM451439), *Bacillus sp.* (HM45431), *Bacillus megaterium* (HM451443), *Bacillus flexus* (HM451429) and *Comamonas sp.*(HM451426) respectively. All the 46 isolates were also tested in static condition (microaerophilic) in nutrient broth. In this experiment, bacterial strain UBL 27 decolorized the wastewater to a maximum of 57.67±0.61 %. This isolate was one among the six isolates that performed well in shaking condition. The other five isolates UBL02, 03, 04, 23 and 24 produced decolorization to similar levels in that of the shaking condition. Based on the findings, UBL-27 was chosen for optimization studies. Experiments revealed that UBL 27 was the best decolorizer for the wastewater (73.2 %) and worked best at pH 8, 30^{o}C in static condition (data not shown).

The difficulties encountered in the wastewater treatment resulting from dyeing operations lies in the wide variability of the dyes used and in the excessive color of the wastewater/effluents (Machado et al, 2006). Thus, in spite of the high decolorization efficiency of some strains, decolorizing a real industrial effluent is quite troublesome (Wesenberg et al, 2003). This is evident in the present study. Bacterial isolates such as UBL01, 02, 03, 43 and 45 that demonstrated significantly high capacityto decolorize the disperse group of dyes (data not shown), could not show similar efficiency in decolorizing the real-time textile wastewater/effluent from the same industry (United Bleachers Pvt. Ltd, Mettuppalayam, Tamil Nadu, India). However, decolorization using fungal culture has greater disadvantages due to 'blanket of biomass' (Overgrowth of mycelial growth) and in further downstream processing of the wastewater. For the convenience and effective management of the treatment plant, use of bacteria is greatly admired.

Physicochemical status of the wastewater samples of

United Bleachers Pvt. Ltd revealed a reasonably high load of pollution indicators compared to the prescribed standards of Pollution board (Banat et al, 1996) (data not shown). There was a gradual change in the color from dark brown to light brown of the textile wastewater from the source (collection point at the textile industry) to the 'Lab-sink' (a temporary storage tank for textile wastewater transport and usage in the laboratory) indicating sign of decolorization. The decreasing color intensity of the wastewater has been related to absorption / chemical transformation of dyes (including metal complex by biotic or abiotic components of the wastewater/effluent) (Blanquez et al, 2004). The increasing bacterial count at the 'lab-sink' might have been responsible for such color change in the present study.

Initially the temperature of the wastewater generated from UBL was considerably high, however, declined to mesophilic status (30°C) at the 'lab-sink', which ultimately has favored biologically mediated remediation of effluent. This was supported by the finding through optimization (data not shown) where 30ºC was found to be the optimum temperature for maximum decolorization of the isolates. This is in consistency with the findings of Asgher et al, (2009); Muhammad et al,(2009); and Swamy and Ramsay, (2007). The trend in decolorization decreased above and below 30^0C. Incubation temperature is a very critical process parameter which varies from organism to organism and slight changes in temperature may affect its growth and ultimately its enzyme production. Higher temperatures may inhibit the growth of organism and enzyme formation which is responsible decolorization (Asgher et al,2009). Bioremediation at higher temperature (40°C) reduces solubility of gases in water that ultimately express as high BOD/COD. This increase in temperature reduced the biodecolorization by almost 10% at 40°C than that at 30°C. High values of BOD/COD as observed in present case demands significant amount of dissolved oxygen for enhanced intrinsic remediation of wastewater. Generally alkaline pH of textile wastewater/effluent is associated with the process of bleaching (Banat et al, 1996) and it is extremely undesirable in a water ecosystem. Both chemically and biologically mediated adsorption/reduction of dyes are initiated with decreasing pH level under redox-mediating compounds (Van der Zee et al, 2003). Decrease in pH i.e., from 10.2 to 8.0 of the effluent significantly improved bacterial count and thereby associated remediation. This is consistent with the findings of Naeem et al, (2009). Total Suspended Solids and Total Dissolved Solids in effluents correspond to filterable and non filterable residues, respectively. Reduction in pH for bioremediated favored microbial growth and the lattereventually resulted in increased in flocculation contributing to the rise as TSS. Microbial community (both aerobic and anaerobic) establishes itself in granulated floc as activated sludge plays a vital role in biodecolorization /bioremediation of wastewater (Lin and Liu, 1994; Lin and Peng, 1996). In the present study, among the 46 bacterial isolates screened for the bioremediation process, only a few isolates show potential decolorizing abilities though of varying degrees under shaking and non shaking conditions. A detailed physiological understanding of such microbes is much needed for bioremediation technology in future.

There has been a strong global awakening during the last few decades regardingthe proper management of existing natural resources. Among them, irrigationwater is one which is becoming costlier due to increasing demands of humanpopulation. Simultaneously the demand for food is also increasing, which has brought moreand more land under cultivation and focused the attention on fertilizer and irrigationwater. With these certain limitations, one has to turn to non-conventional recourses tomeet the irrigation water demand. Among others, one of the most important irrigation aswell as nutrient resources is industrial wastewater, which consists of about 95% waterand the rest as organic and inorganic nutrients. Since, its disposal is a big problem in urbanareas, applying the textile wastewater to agricultural fields instead of disposing off in lakes

and rivers can make crops grow better due to the presence of various nutrients like N, P, Ca, Mgetc. (Kannan et al,2005 and Khan et al, 2003).Use of untreated and treated textile wastewater for agriculture purpose has direct impact on the fertility of soil (Jadhav et al, 2010). Therefore, it is of concern to assess the phytotoxicity of the textile dye effluent before and after degradation by any mode of treatment. Seed germination and plant growth bioassays are the most common techniques used to evaluate the phytotoxicity (Saratale et al,2010). Two important edible crop plants (*Brassica nigra L.* and *Cyamopsis tetragonolobus L.)* have been tested for phytotoxicity of the biologically treated effluents in comparison to the raw wastewater/effluent and chemically treated wastewater.

There was a remarkable performance in the germination percentage of mustard and cluster bean seeds under biologically treated wastewater to about 83.66±0.5 % and 81.31±3.24 % when compared to that of the Control (Group I) on day 4 and day 7, respectively (Table 1). From the results, it was obvious that the raw wastewater (Group II) and the chemically treated wastewater (Group III) reduced the seed germination and early growth in both the test plants (Table 1, 2 & 3). Further, biologically treated textile wastewater did not show any inhibitory effect on seed germination. These findings are in accordance with the others in similar studies. Mohammad and Khan (1985) found that industrial wastewater reduced the germination percentage of kidney bean (*Phaseolus aureus*) and lady's finger (*Abelmoschus esculentus*). Dayama (1987), while working with *Cicer arietinum*, reported that even highly diluted industrial wastewater (5% of industrial wastewater) adversely reduced the seed germination as in *Sesamum indicum* (Neelam and Sahai, 1988), *Holchus lanatus* and *Agrostiss tolonifer* (Amzallag, 1999). The reduction in germination percentage in untreated textile wastewater might have been due to presence of high concentration of Ni^{2+}, Cd^{2+}, Pb^{2+}, and other toxic organic compounds that cause a range of cellular toxicities (Kadar and Kastori, 2003). Although the osmotic potential of the wastewater was not recorded, it is also possible that the presence of high amount of salts and organic compounds in untreated textile wastewater reduces the availability of water thereby resulting in reduced germination. This aspect is further supported by the fact that the presence of high salts in water or soil reduces the germination and early growth of plants by salt-induced osmotic stress that varies from species to species as reported by Ashraf (2004). Few years back, Ramana et al. (2002) found that the osmotic potential of the distillery wastewater is higher at higher concentrations, which retards germination of different vegetable crops.

Slight reduction in growth of the vegetable crops irrigated with 100% treated textile wastewater(Table 2 & 3) might have been due to the persistent levels of some heavy metals (Ramana, 2002). This argument supports the findings of Srivastava and Sahai (1987) who reported that irrigation with distillery wastewater reduced the growth of *Cicer arietinum* (Srivastava and Sahai, 1987). Overall, this inhibition in growth and germination may have been due to the presence of heavy metals such as Ni^{2+} and Pb^{2+} that cause toxicity at cellular as well as at the whole plant level (Kadar and Kastori, 2003). Furthermore, presence of heavy metals in the growth medium also causes reduction in uptake of other essential nutrients thereby resulting in reduced growth (Table 3). For example, while working with tomato seedlings, Palacios et al. (1998) concluded that the presence of elevated concentration of Ni in the rooting medium may cause disturbances and imbalances in different essential mineral elements (Palacios et al, 1998).

Photosynthetic pigments such as chlorophyll 'a', 'b' and total chlorophyll were decreased in the Group II and Group III plants compared to that of Control and Group IV (data not shown). A decrease in chlorophyll content may be due to either the inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions (Barcelo and Gunse, 1985; Chandra et al, 2009). Sahai et al. (1983) reported similar observation when *Phaselous radiatious* was treated with distillery wastewater (Sahai et al, 1983). The more adverse effect of raw textile wastewater and chemically treated wastewater on photosynthetic pigments in *Brassica nigra* and *Cyamopsis tetragonologus* can also be due to the damage done by the presence of heavy metals like Ni and Pb to the photosynthetic apparatus, which possesses both chlorophyll a and b (Seregin and Kozhevnikova, 2006). Similar results were found by Krupa et al. (1993) and Sheoranet al. (1990) who observed a reduction in growth and chlorophyll concentration of bean and pigeon pea, respectively (Krupa et al, 1993; Sheoran et al, 1990).Consequently the two crops irrigated with biologically treated wastewater have demonstrated better growth and yield when compared to untreated (Group II) and chemically treated wastewaters (Group III) (Table 4 & 5). These results can be explained in view of the arguments of different scientists that heavy metals such as lead, nickel and cadmium are highly toxic though at relatively low concentration. They interfere in enzyme action by replacing metals ions from metallo-enzymes and inhibit different physiological processes of plants resulting in poor growth and yield parameters (Kadar and Kastori, 2003; Palacios et al, 1998; Seregin and Kozhevnikova, 2006). However, biological treatment revealed better growth and yield parameters due to accumulation of such heavy metal ions by microbial uptake mechanisms (Ahluwalia and Goyal, 2007). Though, chemical treatment is being currently carried out in the industries that are under pressure to reduce pollutant load, the water that is drained into the streams is not significantly improved in qualities, in terms of toxicity. It is evident in the present study that the chemically treated wastewater is toxic to the plants. However, in biological treatment, though the clarity of the treated water is not to the desired re-use standard due to the biomass accumulation, the detoxification of the treated water is revealed by the phytotoxicity and yield parameters in the plants under study.Since water is a commodity of high demand in the country, with these limitations, one has to turn to non-conventional resources to meet the irrigation-water demand. Since the disposal of untreated textile wastewater is a major problem in urban areas, applying the bioremediation to textile wastewater can make crops grow better due to presence of various nutrients such as N, P, Ca and Mg (Kannan et al, 2005; Khan et al, 2003). Based on the above results, treatment of wastewater can be considered as an effective method in reusing the wastewater from industry for irrigation purposes.

4. Conclusion

In times gone by, staple food production has been dependent on irrigation, and irrigated production is estimated to account for 60% of the world-wide agricultural output. This also holds for India where the Green Revolution was responsible for countering the country's food deficit and was largely been successful due to ground water irrigation. However, currently, effects of overdrafts, like premature failure of wells and monsoon, decline in ground water yield and lowering water tables are apparent. This situation is expected to further worsen due to population growth and the increase in the effective demand for ground water by intensive agricultural production. Within this context, improving water use efficiency in face of the increased water deficit in agriculture requires an integrated approach with the strong commitment of all stakeholders (e.g. textile-industrialists, farmers, plant breeding industry and technology developers). The multiple issues related to water and agriculture are too often hampered by the lack of coordination and exchange of information. On the other hand, the enormous volume of water expelled out of the textile industry may help in encountering the water crisis. For this, treatment of wastewater water and elimination of pollutants is crucial for human health and environmental welfare. The proposed integrated approach of using adapted-bacteria in the detoxification and the re-use of wastewater in irrigation allows farmers, who are unable to access the ground water / unable to make necessary investments in tube wells, to meet their irrigation–water demand. The findings in our study are important to guide the government policy towards providing a solution for textile wastewater treatments and improved water recycling for agriculture. Government should facilitate policies encouraging wastewater treatment and re-use by developing a legal framework. The proposed integrated approach would help textile industries to comply with the norms of the Pollution Control Board; maintain the existing industrial infrastructure contributing to the Indian economy; reduce or eliminate solid/chemical waste deposition in the form of sludge out of chemical treatment and finally encourage industries to develop and maintain a greener environment by diverting biologically treated wastewater water into fields for irrigation, ensuring sustainability of the ground water. There is still plenty of scope for research in this area to improve and optimize the current methods of wastewater treatment. The increased attention to this topic will improve the health, economic and agricultural factors of a sustainably developing community.

Table 1. *Percentage of germination in Brassica nigra and Cyamopsis tetragonolobus in different treatment groups.*

Germination Index (%)	**Group I**	**Group II**	**Group III**	**Group IV**
Brassica nigra	91.66±2.51[a]	36.33±1.52[b]	29±3[c]	83.66±5.03[a]
Cyamopsis tetragonolobus	87.27±1.52 [a]	32.27±2.17 [b]	30.12±0.67[b]	81.31±3.24 [a]

Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)

Table 2. *Mean values of total height; root and shoot length; wet and dry weight in Brassica nigra on day 12.*

Groups/ Parameters	**Group I**	**Group II**	**Group III**	**Group IV**
Total Height (cm)	13.3±0.5[a]	5.9±0.25[b]	4.93±0.2[c]	11.23±0.37[d]
Root Length (cm)	1.98±0.37[a]	1.47±0.15[a]	1.37±0.26[a]	1.71±0.25[a]
Shoot Length (cm)	11±0.1[a]	4.15±0.05[b]	3.33±0.23[c]	9.1±0.2[d]
Wet Wt. (g)	2.1±0.15[a]	0.92±0.035[b]	0.78±0.056[c]	1.52±0.04[e]
Dry Wt. (g)	0.28±0.32[a]	0.13±0.015[b]	0.09±0.005[c]	0.19±0.02[d]

Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)

Table 3. *Mean values of the root and shoot length; height; wet weight in Cyamopsis tetragonolobus under different treatments.*

Groups/ Parameters	**Group I**	**Group II**	**Group III**	**Group V**
Total height (cm)	123±4.58[a]	75.6±3.05[b]	70.3±1.52[b]	102.6±5.03[c]
Root Length (cm)	26±2[a]	19.3±1.52[b]	18.6±1.52[b]	21±2.64[a,b]
Shoot Length (cm)	97±2.64[a]	56.3±1.52[b]	51±2[c]	81.6±2.51[d]
Wet Wt. of Plant (g)	260.3±5.13[a]	130±5[b]	124.3±4.04[b]	227.6±2.51[c]

Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)

Table 4. *Length and Weight of individual pods, Yield/plant in Cyamopsis tetragonolobus among treatment groups*

Groups/ Parameter	**Group I**	**Group II**	**Group III**	**Group IV**
Pod length(cm)	16.3±0.258[a]	13.1±0.1[b]	12.9±0.15[b]	15.7±0.32[d,a]
Weight of individual pod (g)	5.97±0.08[a]	4.04±0.14[b]	3.73±0.19[b]	5.58±0.24[c,a]
Yield /plant (g)	181.57±3.75[a]	85.9±1.71[b]	75.1±4.57[c]	173.98±8.12[e,a]

Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)

Table 5. *Gross yield in Cyamopsis tetragonolobus among various treatment groups in the field study*

Groups/ Parameter	Group I	Group II	Group III	Group IV
Total Yield (Kg)	3.15±0.09[a]	1.84±0.06[b]	1.64±0.07[c]	2.92±0.09[d]

Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)

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