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Research Article

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Stimulatory Effect of Bacteriologically Treated Yamuna River Water on Plant Growth *In vitro*

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ABSTRACT

The present research work deals with the assessment of pollution level in the Yamuna River water. Through this study an attempt has been made to analyze the physico-chemical parameters of untreated and bacterial consortium treated Yamuna river water and its effect on seedling growth of Cajanus cajan. The bacterial consortium treated water increased the germination percentage, shoot length, root length, shoot biomass and root biomass while in untreated water a significant decrease of growth was noticed. Among the different concentrations (25 %, 50 %, 75 % & 100 %) of Yamuna river water, 25 % concentration of untreated water & 100 % concentration of bacterial consortium treated water sample showed stimulatory effect on the seed germination & other growth parameters of Cajanus cajan over control. The result of the study indicates that Effective bacterial consortium helps in the reduction of water impurities and can be effectively used for irrigation.

Keywords: *Bacterial consortium; Germination percentage; Seedling growth; Cajanus cajan; Yamuna River water*

Abbreviations: *EM: Effective Microbes; DO: Dissolved Oxygen; COD: Chemical Oxygen Demand; BOD: Biological Oxygen Demand; TDS: Total Dissolved Solids; TSS: Total Suspended Solids; TS: Total Solids; mg* L^{-1}*: milligrams per Litre; g: grams; cm: centimeters; APHA: American Public Health Association;* $HgCl_2$*: Mercuric Chloride*

INTRODUCTION

Everything originated in the water, and everything is sustained by water. Water is one of the essential materials required to sustain life and unfortunately has long been suspected of being the source of human illness[1]. India is facing a serious problem of natural resource scarcity, especially that of water in view of population growth and economic development. Water is a prime natural resource, a basic human need and a precious national asset and hence its use needs appropriate planning, development and management[2].

The status of the Yamuna river water is very much useful as it determine the physiological life cycle of plants, animals and human kingdom. In Agra (city of Taj) the Yamuna River has always been the most important fresh water resources. However the Yamuna water is used in every sector of development like agriculture, industry, transportation, aquaculture, public water supply etc. Huge load of wastes from industries, domestic sewage and agriculture practices find their way into the Yamuna, resulting in large scale deterioration of the water quality and affect the physico-chemical parameters of water.

Microorganisms cleaned the Earth in its early stages when it was polluted, helped create the oceans, rivers, and soil, and helped maintain an environment in which living things could thrive.

The concept of Effective Microorganisms was developed by Japanese horticulturist Teuro higa from the University of Ryukyus in Japan.

He reported in the 1970s that a combination of approximately 80 different microorganisms is capable of positively influencing decomposing organic matter such that it reverts into a life promoting process[3]. The Studies have shown that EM may have a number of applications, including agriculture, livestock, gardening landscaping, composting, bioremediation, cleaning septic tanks, algal control and household uses[4]. The application of EM will improve soil and irrigation water. It can be used in seed treatment. However, in biological treatment, the microorganisms degrade the organic pollutants using them as a carbon source to produce metabolic energy to survive. The effects of various industrial effluents, sludge materials and metal elements on seed germination, growth and yield of crop plants have captivated the attention of many workers[5-7].

Hence an attempt has been made to study the effect of the Yamuna river water (both treated and untreated) on germination and seedling growth of *Cajanus cajan* in vitro.

EXPERIMENTAL PROTOCOL

Sample Collection

Water samples were collected in pre-sterilized BOD bottles, from the river Yamuna situated in the city of Taj - Agra, India, receiving the huge loads of industrial, agricultural and domestic wastes resulting in large scale deterioration of the water quality and affects the physico-chemical parameters of water. Samples were stored and maintained at 4°C for further experimental analysis.

Isolation of Bacterial Isolates

The bacterial isolates present in the Yamuna River water were isolated by Serial dilution (Pour-Plate) technique[8]. For the selected isolation of bacteria Nutrient agar media were used. Nutrient Agar medium was prepared by mixing Peptone-5 g, Beef extract-3 g, Sodium chloride-5 g, Agar-20 g, pH-7 in 1000 ml distilled water. After the incubation period the plates were observed for growth on the media[9]. When the colonies appear on agar plates, each one was sub cultured on a new agar plate until pure strains were obtained.

Optimization for growth profile analysis

The ability of the bacterial isolates was tested in five different broth media (Sucrose broth, Nutrient broth, Yeast Extract Broth, Trypticase Dextrose broth, Trypticase soya broth) followed by the methodology of Augustine[10]. The optical density of the uniform suspension was measured at 620 nm using UV-vis spectrophotometer (Systronics-2203).

Identification of selected Bacteria Isolates

The isolated bacterial cultures were identified on the basis of their morphological, physiological and biochemical characteristics features by Bergey's Manual of Systematic Bacteriology[11]. These cultures were further cross examined by BD-BBL Crystal Identification Autoreader (Becton Dickinson and Company, USA) for the identification surety.

The BBL CRYSTAL RGP ID panels contain 29 dried biochemical and enzymatic substrates. A bacterial suspension in the inoculum fluid is used for rehydration of the substrates. The tests used in the system are based on microbial utilization and degradation of specific substrates detected by various indicator systems[12].

Physico-chemical analysis of Yamuna waste water

The analysis of initial physico-chemical parameters such as DO, BOD, COD, TDS, TSS, TS, acidity, alkalinity and hardness of collected Yamuna water samples were carried out by standard methods[13].

Bacterial Consortium preparation

Bacterial cultures (*Rhodopseudomonas palustris*, *Rhodobacter spheroides*, *Escherichia coli*, *Bacillus subtilis*, *B. cereus*, *B. thuringiensis*, *B. fusiformis*, *Lactobacillus* sp) were inoculated individually in pre-sterilized 100ml Nutrient broth. The flask was kept in a shaker at 120 rpm for 16 to 18 h at 30°C. The culture broth was centrifuged at 10000 rpm for 20 min. Cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD using UV-visible spectrophotometer[14]. 5% of the above stock solution of Effective Microorganisms was added in Jaggary Solution (Jaggary-100g, Yeast extract-10g, Distilled water- 1000ml pH-6.5.

Dissolve all the ingredients in distilled water and autoclaved at 121˚C at 15 lbs for 15 min.).The inoculated Jaggary medium was incubated at 37˚C temperature for 5 days.

Efficiency of bacterial consortium with respect to the reduction of physico-chemical properties of Yamuna waste water

The efficiency of the bacterial consortium was evaluated with respect to changes in physico-chemical parameters of the wastewater samples after treatment by following the standard method[13]. For bacterial consortium treatment, 5% of EM solution containing bacterial consortium was inoculated into the water samples and incubated in an incubator. After incubation, the samples were analyzed for different physico-chemical parameters.

Effect of treated and untreated Yamuna water sample on seed germination (*Cajanus cajan*) in vitro

In *in vitro* studies the seeds of *Cajanus cajan* were collected from Dayalbagh Agriculture Farm, Dayalbagh, Agra, (India) and petriplates of 180 cm were used in this experiment. The seeds were sterilized with 1% $HgCl_2$ solution for two minutes and washed with sterilized distilled water 2-3 times to remove the contamination of seed coat prior to germination study. Twenty seeds were placed at an equi-distance in sterilized petriplates lined with Whatman Filter paper No. 41. Known volume of different concentration (25%, 75%, 50% and 100%) of untreated and bacterial consortium treated waste water samples were poured into different petri-plates. Each treatment including the control was run in triplicates. Plates were kept in an incubator and maintained under standard aseptically physiological conditions at 25-27˚C temperature, 16 hours light and 8 hours dark cycle. After alternate days the fixed amount of different concentration of untreated and bacterial consortium treated water samples was poured into plates upto7 days.

RESULTS AND DISCUSSION

From the samples collected during January 2012 to August 2012, total 15 bacterial strains were obtained from the samples. (Fig.1). The bacterial isolates having highest prevalence i.e. 100% throughout were selected and identified on the basis of their morphological, physiological and biochemical characteristics (Table-1). After biochemical test for confirmation of the identified bacteria, a new technique of BD-BBL Crystal Mind Auto reader was also used for rapid identification of bacteria. It showed results between 95 to 99% purity of identified bacteria. The bacteria were tentatively identified as:- **B1-***Rhodopseudomonas palustris,* **B2-***Rhodobacter spheroides,* **B3-***Escherichia coli,* **B4-***Bacillus subtilis,* **B5-***Bacillus cereus,* **B6-***B. thuringiensis,* **B7-***B. fusiformis,* **B8-***Lactobacillus* sp.

The growth of the potent eight bacterial isolates were observed in five different growth media i.e. Nutrient Broth (NB),Trypticase dextrose broth (TDB),Trypticase Soya broth (TSB),Yeast Extract broth (YB) and Sucrose broth at 37°C for 24, 48 and 72 hours of incubation period. It was observed that all the eight bacterial isolates showed maximum growth 3.222, 3.026, 3.035, 3.283, 2.905, 3.217, 3.283 and 3.283 in Sucrose broth. The bacterial isolates showed moderate growth in TDB, TSB & YB medium and minimum growth 2.412, 2.448, 1.909, 1.343, 2.47, 2.416, 1.949, 2.017 in NB medium. The growth was increased up to 48 hours and becomes constant upto 72 hours (Fig 2). Saini[15] observed the growth of the six bacterial isolates (*Rhodopseudomonas palustris, Rhodobacter spheroids, Bacillus subtilis, Lactobacillus* species & *Nitrobacter* species) in five different broth media i.e. NB, TDB, SB,TSB & YB medium at 37°C for 24,48 & 72 hours of incubation period. All the six bacterial isolates showed maximum growth in TSB medium upto 48 hours and become constant with increasing the incubation period (72 hours).

The physico-chemical characteristics of Yamuna waste water before and after treatment are presented in Table 2. All the physico-chemical parameters except DO showed maximum values in untreated Yamuna water sample but the bacterial consortium treated showed much reduction in the physical and chemical parameters to the way of improvement. These findings can serve as an important contribution towards an economic and simplified the biological methods for the waste water treatment using bacterial consortium. Synergistic association of specific aerobic and facultative bacteria able to use a wide range of organic contaminants reduced the physico-chemical parameters of waste water. Saini[16] reported that the physico-chemical parameters of untreated domestic waste water were beyond the tolerance limit but after treatment with bacterial consortium all the parameters were sharply reduced, below the tolerance limit.

The results are similar with the report of Chandra[17] revealed that the efficiency of active bacterial consortia (*Pseudomonas putida*, *Rhodopseudomonas*, *Citrobacter*, *Enterobacter*, & *Rhodobacter*) in removal of the colour and reduction of BOD, COD, TDS, TS and hardness.

Ayyasamy[18] supported the present findings. They used different combinations of bacterial and fungal cultures and observed that consortium of five bacterial strains (*Alcaligenes* sp, *Corynebacterium* sp, *Micococcus* sp, *Bacillus* sp, *Pseudomonas* sp.) showed the maximum percentage reduction in BOD, COD and other parameters of effluent in comparison to single and dual bacterial cultures.

Kumar and Bhoopathi[19] also supported the present result and reported that the consortium of different bacterial species (*Pseudomonas* sp, *Cellulomonas* sp, *Alcaligenes* sp) showed the significant reduction in different parameters of sago factory industry.

Fig. 1: Frequency of different bacterial colonies in samples collected from January 2012 to August 2012

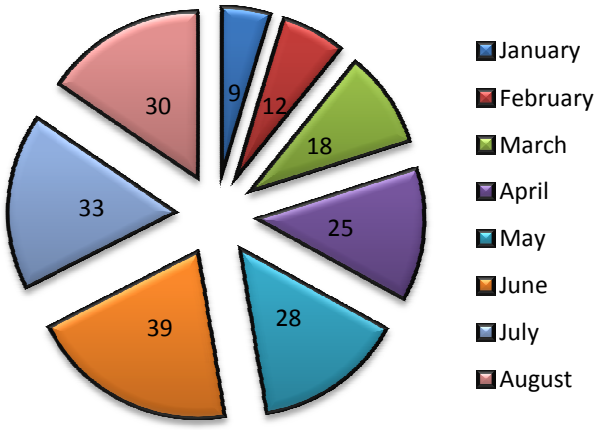


Fig. 2: Effect of differerent broth medium on the growth of bacterial isolates after different incubation period

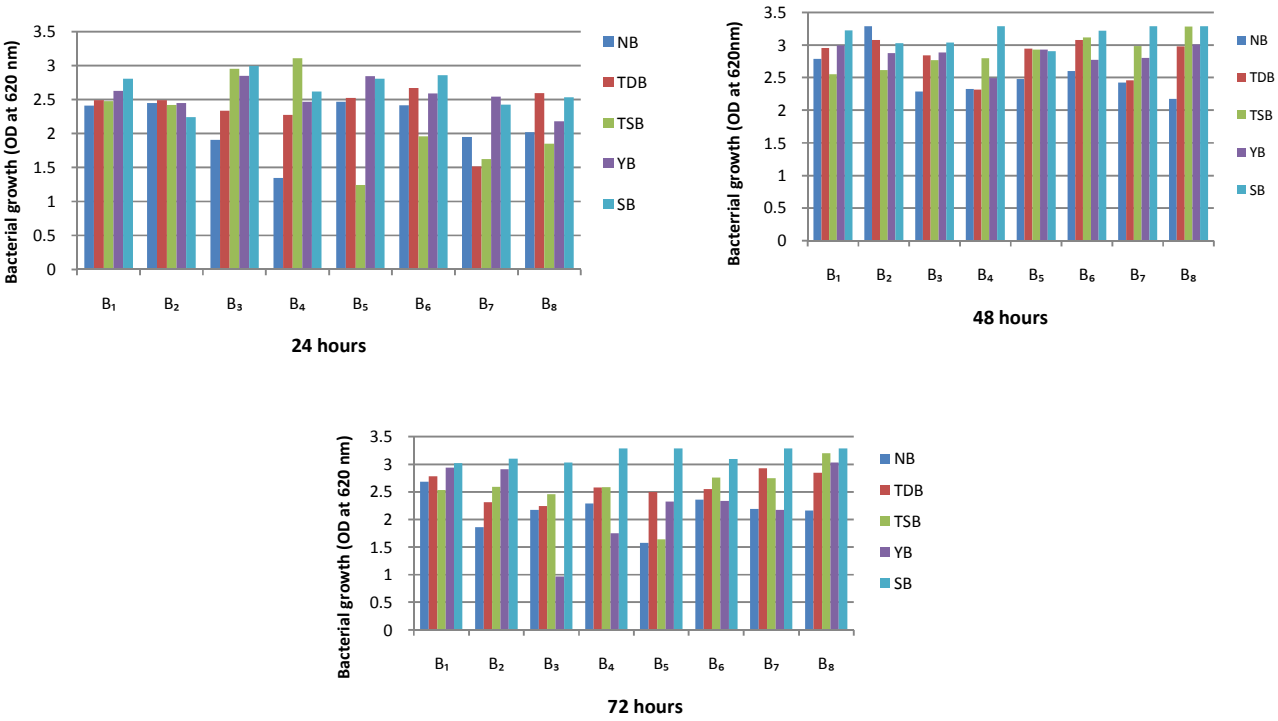


Table 1: Biochemical characteristics of isolated bacterial isolates

Characterization	B1	B2	B3	B4	B5	B6	B7	B8
Gram Staining	Gram -ve	Gram -ve	Gram-ve	Gram +ve	Gram +ve	Gram +ve	Gram-ve	Gram+ve
Motility	Motile	Motile	Motile	Motile	Motile	Motile	Motile	Motile
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Thin Rod
Oxygen Req.	Aerobic	Aerobic	Anaerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Colony	Red	Creamy white	Greyish	Orange-yellow	Dull cream	White cream	Greyish white	White
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	+	-	+	+	-	+	-
Urease	-	-	-	-	-	+	-	-
H_2S production	-	-	-	-	-	+	-	+
Indole production	-	-	+	-	-	-	-	+
M R Test	+	+	+	-	+	+	-	-
V P Test	-	-	-	+	-	+	-	+
Citrate Utilization	+	+	+	+	-	-	+	+
Glucose	+	+	-	-	+	+	+	+
Mannitol	-	-	-	+	-	-	-	+
Lactose	+	+	+	-	-	+	-	+
Sucrose	+	+	-	-	-	-	-	+
Fructose	+	+	-	+	-	+	+	+

***B1**-*Rhodopseudomonas palustris*, **B2**-*Rhodobacter spheroides*, **B3**-*Escherichia coli*, **B4**-*Bacillus subtilis*, **B5**-*Bacillus cereus*, **B6**-*B. thuringiensis*, **B7**-*B. fusiformis*, **B8**-*Lactobacillus* sp.

Table 2: Physico-chemical characteristics of Yamuna water before and after treatment with bacterial consortium

Physico-chemical parameters	**Untreated***	**Treated***	**Percentage reduction (%)**
Colour	Brown	Colourless	-
Odour	Foul smell	Odourless	-
pH	8.9	7.1	-
Acidity (mg L^{-1})	498.7	79.3	84
Alkalinity (mg L^{-1})	1197	124	90
Hardness (mg L^{-1})	1834	524	71
TDS (mg L^{-1})	56.8	7.0	88
TSS (mg L^{-1})	32.9	4.9	85
TS (mg L^{-1})	23.9	2.1	91
DO (mg L^{-1})	5.6	78.9	93
BOD (mg L^{-1})	29.9	3.3	89
COD (mg L^{-1})	152.9	24.3	84

*mean±standard deviation from three replicates

In case of seed germination, the effect of untreated and treated water on *Cajanus cajan* is presented in Table 3, Fig.3. In *in vitro* study on *Cajanus cajan* the growth parameters were observed after 7 days. In untreated water sample, the lower concentration (25%) proved to be very efficient in germination percentage and seedling growth, shoot and root biomass of *Cajanus cajan.* These findings are similar with the report of Mythili and Karthikeyan[14] reported that the higher concentrations (80 and 100%) of tannery effluent suppressed the germination of blackgram and sunflower. It may be due to the effect of higher amount of total solids and heavy metals stress on the seed germination process in untreated effluent.

Paliwal[20] and Singh[21] observed that high concentrations of waste water caused the reduction of seedling growth due to the presence of high amount of complex organic and inorganic matter and excess of various forms of cations and anions which on coming in contact with germinating seed may enter the body system, resulting in ultimate damage to the seed. The results are in accordance with the report given by Verma[22] that the higher concentration of the waste water contains more pollution load, which causes deleterious effect on crop production.

The results are also similar with the findings of Francosis and Mass[23] who reported that adverse effect of 100% concentration of untreated water on plant growth may be due to the influence of ions in waste water in large quantity which makes them toxic to the seed embryo. Our results are in concordance to that of Kannabiran and Pragasam[24]. They showed that the negative impact of higher concentration of waste water on seed germination of *Vigna radiata* might be due to the higher contents of dissolved solids, which prevent the germination by contributing the salinity of the solute absorbed by seeds before germination.

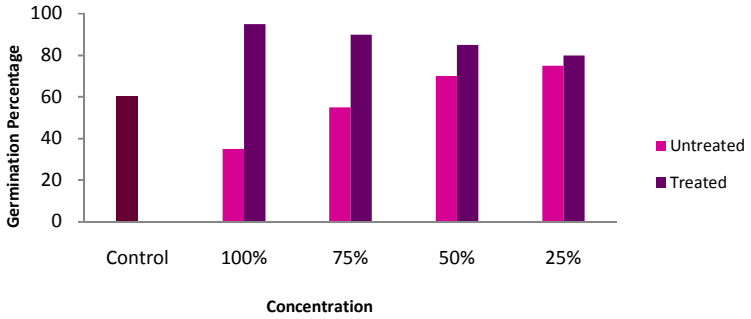
Similar findings were reported by Kumar and Bhoopathi[19] that the bacterial consortium (*Alcaligenes* sp *Bacillus* sp and *Pseudomonas* sp) treated sago factory effluent better response in seed germination and seedling growth of *Vigna radiata* and *Vigna munga* in comparison to untreated and control. Similarly Daly and Stewart[25] reported that application of Effective Microorganisms (EM) to onion pea and sweet corn increases their yield.

Table 3: Effect of untreated and bacterial consortium treated Yamuna water sample on the growth of *Cajanus cajan in vitro*

Concentration	Shoot length (cm)	Root Length (cm)	Shoot Biomass (g/plate)	Root Biomass (g/plate)
	Mean ± S.D	Mean ± S.D		
Control	6.4 ± 2.1	5.4 ± 1.6	1.49	1.23
Untreated				
100%	9.2 ± 2.2	3.4 ± 1.1	1.62	1.39
75%	9.7 ± 3.5	4.6 ± 1.8	1.83	1.42
50%	11.0 ± 1.1	5.2 ± 2.2	1.85	1.54
25%	13.2 ± 2.1	5.7 ± 2.1	1.93	1.69
Treated				
100%	13.7 ± 0.7	8.9 ± 0.2	2.81	1.93
75%	13.4 ± 1.8	8.5 ± 0.5	2.31	1.85
50%	12.9 ± 0.8	7.3 ± 1.3	2.22	1.81
25%	12.3 ± 2.5	6.8 ± 2.1	2.14	1.78

*mean±standard deviation from three replicates

Fig. 3: Effect of untreated and bacterial consortium treated Yamuna water sample on Germination Percentage of *Cajanus cajan in vitro*



CONCLUSION

In the growing awareness of relationships between human health and water pollution, it is essential to undertake regular monitoring and surveillance of important aquatic ecosystems. The results of present study indicate that EM technology helps in the reduction of water impurities. The observation revealed that the inoculation of bacterial consortium in water may release the nutrients through biodegradation of the organic/inorganic matter of water sources, which promote the plant growth. Moreover, the regular monitoring of water pollution level of river basin, appropriate purification treatment and community participation in water resources management will certainly help managers in taking informed decisions for water resources sustainability and management.

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