

**Research Article**

## **BIOREMEDIATION OF PAPER MILL EFFLUENT ON GROWTH AND DEVELOPMENT OF SEED GERMINATION (*VIGNA MUNGO*)**

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### **ABSTRACT**

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization. The study was concerned about the effect of the paper mill effluent in the agricultural fields as an irrigation perspective. *In vitro* experiment was done where the seeds of *Vigna mungo* were treated with the different concentrations of untreated and bioremediation treated paper mill effluent. The effect of effluent on seedling growth was suitable in the lower concentration (40%), whereas the higher concentration (80, and 100%) was inhibitory in untreated. The paper mill effluent after treatment was found to be of improved quality and showed good germination and seedling growth.

**Keyword:** Paper Mill, *Vigna Mungo*, Germination Percentage, Total Seedling Length (cm), Vigour Index

### **INTRODUCTION**

Urban industrial activities have long been identified as a major source of environmental pollution. Now-a-days, treated wastewater is considered as a potential water resource because it contains considerable amount of nutrients, which may prove beneficial for plants growth (Sahai *et al.*, 1985; Mishra and Behera, 1991) and hence the use of wastewater in agriculture is gaining importance rapidly. Generally, the quality of discharged effluent differs from industry to industry, which may or may not be suitable for the irrigation of crop. So, the effluent should be assessed properly prior to its application for irrigation. However, indiscriminate use of industrial effluent may reduce crop growth and contaminants may interfere with natural characteristics of soil.

Rajannan and Oblismai (1979) reported that paper mill effluent had drastically affected the germination of rice, black gram and tomato seeds, however, the diluted form of effluent (25 and 50%) enhance the growth. Karande and Ghanvat (1994) observed that dilute effluent show negligible effect on the overall growth characteristic in pigeon pea seedling treated with paper mill effluent. Mishra *et al.*, (1989, 1991) conducted the study of phytotoxicity of the paper mill effluents on *Elusine coracana* and *Oryza sativa* crops.

Further, Mishra and Behera (1991) studied the same effect on rice seedlings. Narwal *et al.*, (2005) reported that paper mill effluent increased sodium and potassium contents and disturbed the anionic-cationic balance in plants, thereby reducing the yield and quality of crops. However no detailed experiments have been performed on the germination and plant growth using treated and untreated tannery effluents. Hence an attempt has been made to study the effects of paper mill effluent (both treated and untreated) on the germination of blackgram.

### **MATERIALS AND METHODS**

#### ***Effluent Source***

The effluent was obtained from RAJAGANAPATHY PAPER MILL (Board mill private ltd) VADAMANGALAM, Pondicherry, A south Indian base integrated pulp and paper mill industry. The effluent was collected from outlet of primary settling tank were used for investigation. The sample was collected in a plastic container and transported to Annamalai University Microbiological Research laboratory situated in Chidambaram within 4 hours. The effluent was stored at 4°C until further investigation.

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### **Bacterial Isolation**

The sample was serially dilution using sterile pipettes from  $10^{-1}$  and  $10^{-8}$  dilution the bacterial strain capable of growing on nutrient agar medium. For enumeration of bacteria nutrient agar medium was used. To obtain pure culture were repeatedly streaked nutrient agar medium and incubated at  $37^{\circ}\text{C}$  for 24 hours. The isolated bacteria were identified by colony morphology, gram staining, microscopic observation and confirmation test. The identified bacteria were, *shigella sonnei*, *Pseudomonas aeroginosa*, *Bacillus subtilis*. The isolated fungal culture was identified as *Trichoderma reesei* using Lactophenol cotton blue staining method.

### **Physico-Chemical Parameters Assessed in Bioremediated Paper Mill Effluent**

Based on the bioremediation process, effective method was used to treat the paper mill effluent. After remediation, the physico-chemical parameters were assessed followed by the earlier said effluent analysis procedures.

### **Preparation of Inoculum**

A loopful of culture (*shigella sonnei*, *Pseudomonas aeroginosa*, *Bacillus subtilis*.) was inoculated individually pre-sterilized 100ml nutrient broth. The flask was kept in a shaker at 120rpm for 16-18hrs at  $30^{\circ}\text{C}$ . The culture broth was centrifuged at 1000rpm for 20 minutes. cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD using UV-spectrophotometer, 1% ( $10^5$  CFU/ml) of the above suspension was used as inoculum for the bioremediation of paper mill effluent.

### **Seed Germination and Plant Growth Experiments**

In order to check the plant growth ability of the raw and bioremediated paper mill effluent, the growth experiments were carried out with economical important cash Blackgram (ADT-3) crop (*Vigna mungo*) in laboratory conditions. The present study various dilutions of the selected paper mill effluent (both untreated and bioremediated) were used for plant growth study and observation were recorded.

### **Seed Collection**

The seeds of Blackgram (ADT 3, Duration: 75 days) seed were collected from experimental farm, Department of Agronomy, Faculty of agriculture, Annamalai University, Tamil Nadu, India.

### **Seed Germination Experiment under invitro Condition**

Germination studies were conducted under laboratory conditions. Six test solutions ( $T_0=0\%$ ,  $T_1=20\%$ ,  $T_2=40\%$ ,  $T_3=60\%$ ,  $T_4=80\%$  &  $T_5=100\%$  v/v) prepared by diluting paper mill effluent for both untreated and bioremediated with distilled water were used to investigate the effects of wastewater on germination of blackgram. The healthy and uniform sized blackgram (ADT-3) seed were surface sterilized with 0.1% mercuric chloride for 2- 3 minutes, washed in running tap water for 3 minutes and in distilled water for 2 minutes. They were thoroughly washed with tap water to avoid surface contamination. Ten healthy and undamaged seeds of equal size were evenly placed in each sterilized petridish which contained water soaked filter papers.

The petridishes were arranged in completely randomized block design. Measured quantities of test solutions was added in each replicate and exposed for 8 hours. Seedlings were then grown in distilled water. Random samples were taken from each treatment after 7 days. Following growth parameters were measured and recorded.

### **Growth Measurements under in vitro Condition**

- Seedling length
- Germination percentage
- Total seedling length (cm)
- Vigour index

### **Germination Percentage**

The number of seeds which germinated after sowing was counted and the percentage was calculated.

### **Seedling Length**

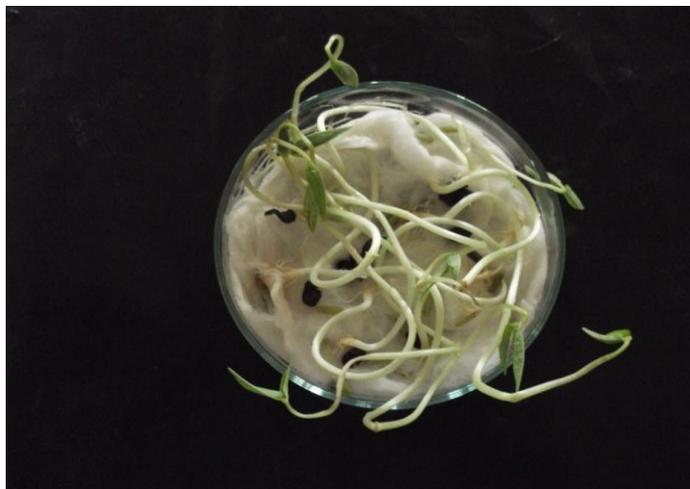
On the eighth day, the seedlings from different concentrations were removed carefully and washed in water. The length of root and shoot was measured with the help of a wetted twine (for flexibility) and a scale.

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#### **Vigour Index**

Vigour index was calculated by the formula

V.I. = Germination percentage x total length of seedling (cm)



**Plates 1: Seed germination of blackgram at 40% bioremediated paper mill effluent**



**Plates 2: Seed germination of blackgram at 100% untreated paper mill effluent**

### **RESULTS AND DISCUSSION**

The effect of untreated and bioremediated paper mill effluent on seed germination, seedling length and the vigour index of blackgram given in table-1. The seeds grown in control and 20% of untreated effluent showed the highest germination of 100% and its vigour index was 860, 900 respectively. Whereas in the bioremediated effluent upto 40% concentration was recorded 100% germination (plates-1 and 2), and three after that it was gradually decreased. The vigour index at 20, 40 per cent of the bioremediated effluent was increased over the control with 940, 960 respectively. Seeds of blackgram grown with 60, 80 and 100% untreated effluent showed a reduction in vigour index (490, 375 and 230) compared to the control plant. The seedling length showed an increase over control upto 40% concentration of bioremediated effluent and slightly decreased in the higher concentrations.

The experiments were conducted to study the effect of untreated and bioremediated paper mill effluent on the growth and yield of blackgram (ADT 3) is an important crop of this area, so as to know the possible use of paper effluent as a source of water for the purpose of irrigation. The present work is concerned with finding out the optimum concentration of the paper mill effluent and its bioremediation for the better

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germination and behavior of seedlings on the one hand and more yield of blackgram growing under *in vitro*, pot and field conditions. In the present study, germination percentage, total seedling length, vigour index, total chlorophyll, carbohydrate and protein contents of blackgram were observed under *in vitro* condition. *In vitro* experiment were carried out in the laboratory to find out the morphological and biochemical responses of seedlings of blackgram to five concentrations (20, 40, 60, 80 and 100%) of the untreated and bioremediated effluent compared with control.

The seeds of blackgram showed highest germination percentage, total seedling length and vigour index in bioremediated effluent upto 40% concentrations whereas the have raw effluent high percentage in 20% concentration germination. The effect of paper on germination of seeds was attributed to the presence of biotoxic substances, which alter the seeds the water interaction necessary for triggering enzyme activity Baruah and Das (1997). Among the different concentration of raw effluent upto 40% most suitable for the crops. This earlier report was done by Medhi *et al.*, (2008) 40% concentration for mustard crop. The lower concentrations of paper mill effluent had a marked growth promoting effect on overall growth of seedling, while higher concentration of effluent had inhibiting effect in seed germination, seedling growth the seeds.

The increase in the germination percentage at lower concentration of the effluent undoubtedly indicates the stimulating effect of physiologically inactive seeds by diluted effluent (Biradar *et al.*, 1989). It might also be due to the fertilizing effect of lower concentrations of the effluent and optimum conditions provided for seed germination (Jerath and Sahai, 1982). The stimulation of  $\alpha$ -amylase activity of embryos might also be one of the reasons for enhanced germination percentage (Yadav *et al.*, 1988).

**Table 1: Effect of untreated and bioremediated paper mill effluent on seed germination, total seedling length and vigour index of Blackgram under *in vitro* condition**

Effluent Concentration (%)	Untreated effluent			Bioremediated effluent		
	Germination (%)	Total seedling length (cm)	Vigour index	Germination (%)	Total seedling length (cm)	Vigour index
Control	100	8.6	860	100	8.6	860
20	100	9.0	900	100	9.4	940
40	92	8.9	818	100	9.6	960
60	70	7.0	490	98	7.5	735
80	67	5.6	375	95	6.2	589
100	59	3.9	230	90	5.5	495

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