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Utilization of Handmade Paper Industries Effluent Fortified with Growth-promoting Microbes for Enhancing *Vigna radiata* Growth

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ABSTRACT

Background: Biofertilizers play an essential role in bringing sustainability to agriculture. They are being focused all over the world to develop and improve technologies for biofertilizers to make them cost-effective and enhance productivity. Handmade papermaking from cow dung results in the generation of effluents, raw liquor (RL) and alkaline black liquor (BL), which are rich in organic carbon and nutrients. The present study was carried out to study the compatibility of RL and BL in agriculture along with microbial biofertilizers.

Methods: One of the waste effluents (RL) was recently reported to have plant growth-promoting characteristics with the promising results of seed germination and pot studies using green gram seeds at KNHPI. In the present study, the RL and BL efficacy were tested with microbial cultures procured from different sources and isolated at KNHPI for enhancing their biofertilizer potential. The cultures, namely mung Rhizobium (MR), Phosphate Solubilizing Bacteria (PSB-NB), *Enterobacter kobei* (ECM), *Bacillus* species isolated from soil B.St (S), *Bacillus* species isolated from textile industry waste B.St (W) were selected for detailed study in different combinations.

Result: The combination of RL and BL with different PGPR strains of a compatible nature was observed to show enhanced seed germination and plant growth. The present study provides an insight into the utilization of waste by-products of the handmade paper industry that will have socio-economic implications. This may contribute to bring sustainability to the agriculture sector and help provide employment, income generation and reduce environmental pollution.

Key words: Agriculture, Biofertilizer, Effluents, Environmental pollution, Handmade paper industry, Sustainable development.

INTRODUCTION

The pulp and paper sector is recognized as the sixth largest contributor to industrial pollution, following the oil, cement, leather, textile and steel industries, by releasing diverse contaminants into the environment (Savant *et al*., 2006; Mehmood *et al*., 2019). Papermaking is highly energy-intensive and water-consuming in terms of freshwater utilization (Pokhrel and Viraraghavan, 2004). Water consumption varies with production methods and may reach up to 60 m^3 per ton of paper, generating ~50 m^3 of wastewater (Thompson *et al*., 2001; Ashrafi *et al*., 2015; Molina-Sanchez *et al*., 2018). Freshwater is used at every stage, from raw material cultivation to pulping, bleaching and sheet formation, resulting in substantial wastewater generation (Chauhan and Meena, 2021).

Major pollutants of pulp and paper effluents include lignin, fatty acids, dark colour compounds, biochemical oxygen demand (BOD), chemical oxygen demand (COD), adsorbable organic halides (AOX) and volatile organic compounds (VOCs) (Sharma and Singh, 2021). According to the ministry of environment and forest (MOEF), the pulp and paper industry is categorized under the red category due to its high pollution potential (Patel *et al*., 2021). Wastewater generation ranges from 220-380 m^3 per ton of paper produced and is often highly coloured and toxic (Badar and Farooqi, 2012). In India, total water consumption may reach 250-300 m^3 per ton of paper (Chaudhary and Paliwal, 2018; Patel *et al*., 2021). Various treatment approaches, including aerobic, anaerobic, electrochemical, photocatalytic, ozonation, coagulation-flocculation and adsorption techniques have been investigated (Singh and Tripathi, 2020; Touhari *et al.,* 2023). Biological treatment methods rely on microbes to convert organic pollutants into biomass (Sharma and Singh, 2021). However, efficient treatment depends on wastewater characteristics, quantity and reuse requirements, emphasizing the need for well-defined management strategies (Singh and Tripathi, 2020).

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Handmade paper refers to sheets produced manually through traditional sheet-forming processes. The Khadi and Village Industries Commission (KVIC) includes paper made using Cylinder Mould Machine/Vat (CMM or CMV) with a deckle width of 102 cm in handmade paper category. Handmade paper is considered socially responsible and sustainable as it is tree-free, eco-friendly and resource-efficient (Chauhan *et al.*, 2022).

It is justified as a cost-effective and energy-saving alternative with strong economic and environmental benefits (Reddy, 2015). Although handmade and mill-made paper share similar principle of production, differences exist in processing, energy use and environmental impact (Chauhan and Hussain, 2009; Chauhan *et al*., 2022). Both sectors generate coloured effluents that possess environmental and aesthetic concerns, necessitating proper treatment and disposal.

Kulshreshtha *et al.* (2011) have evaluated the health perspectives of the effluents of the handmade paper industry of the Sanganer region and reported them to be mutagenic with either one strain of *Salmonella typhimurium* or with both. The presence of dyes and chemicals in the effluents of handmade paper and pulp industries is revealed by its colour, low pH and high COD (Kulshreshtha *et al*., 2010a). The handmade paper and cardboard industries also produce sludge, at the rate of approximately 10 kgd^{-1} per industry, in the form of shortened pulp residues which cannot be recycled again. These residues are accumulated in the drain or street and produce enormous odour. Kulshreshtha *et al.* (2013) have reported the cultivation of *Pleurotus citrinopileatus* on the sludge of handmade paper and cardboard industrial waste. Agro-industrial and industrial wastes have been exploited for centuries in Asia for the production of oyster mushrooms (Zervakis *et al*., 1995; Sivrikaya *et al*., 2002; Kuforiji and Fasidi, 2009; Kulshreshtha *et al*., 2010b).

Recycled water is defined as wastewater that is treated and reused to supplement water supply (US EPA, 1992). The beneficial utilization of treated wastewater for agriculture is the major water reuse application worldwide (US EPA, 2004). Research shows that wastewater irrigation can result in significant changes to soil's physical, chemical and biological properties. Kunhikrishnan *et al.* (2012) have reported the influence of wastewater irrigation on the transformation and bioavailability of heavy metal (loid)s in soil. González *et al*. (1992) have explored the possibility of utilizing kraft black liquors generated in a paper mill, using *Eucalyptus globulus* as raw material, for the production of a Nitro-humic soil conditioner. Liu *et al.* (2021) have reported a pulping black liquor-based polymer hydrogel as a water retention material and slow-release fertilizer. Similarly, Haile *et al*. (2021) have reviewed the prospects of making different high-value-added biomaterials from pulp and paper mill wastes.

Therefore, in view of all the above background, the present study was taken up to explore the possibility of improving the use of effluents in the handmade paper industry using cow dung as raw materials. The effluents were augmented with different PGPR strains and evaluated in green gram (*Vigna radiata i.e.* mung). While the utilization of industrial effluents (Baskar *et al*., 2026) and PGPRs (Kajić *et al*., 2025) (in agriculture has been explored independently, this study contributes by systematic evaluation of their combined application, particularly using cow dung-based handmade paper effluents, for improving mung bean growth. Thus the study offers a novel integration of handmade paper industry effluents with plant growth-promoting microbial consortia, providing new insights into their combined potential as eco-friendly biofertilizer formulations for enhancing *Vigna radiata* growth.

MATERIALS AND METHODS

The present study was carried out in the Biotechnology laboratory of Kumarappa National Handmade Paper Institute (KNHPI), Jaipur, Rajasthan during the Financial year' 2021-22.

Procurement of standard microbial strains of PGPR

Pseudomonas sps (PSB-NB) and the bacterial strains of rhizobia, namely mung Rhizobium (MR), chickpea *Rhizobium* (CPR) and pea *Rhizobium* (PR), were procured from the National Botanical Research Institute (NBRI), Lucknow. *Azotobacter sp. (Azo-R)* was kindly received from Krishi Vigyan Kendra, Rajsamand. *Bacillus megaterium* (BM), *Bacillus safensis* (B.*sf*) and *Enterobacter kobei* (ECM) were procured from National Agriculturally Important Microbial Culture Collection (NAIMCC), ICAR-National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM), Mau. Two *Bacillus* species were isolated from garden soil (Bst.S) and textile industry effluents (Bst.W) at KNHPI, Jaipur, Rajasthan.

Collection of waste effluents of handmade papermaking

Two types of waste streams/effluents of cow dung based handmade paper industry were collected. Raw liquor (RL) obtained before pulping of the cow dung squeezed in the dewatering machine and after pulping by NaOH (BL) were used in the study (Narayana *et al*., 2024).

Examining the capability of microbial cultures to utilize RL/BL as the sole source of nutrient

RL and BL plates were prepared separately by adding agar powder (Bacteriological grade, HiMedia, GRM026) at the rate of 3% of the effluents used (RL and BL). The cultures were streaked on RL and BL agar plates. The inoculated plates were incubated at 28°C for 3 to 5 days and the growth of bacterial culture indicated the utilization of RL and BL by the microbes used. Sterilized water agar media supplemented with un-autoclaved RL and BL separately were also included in the study. These plates were inoculated with the available PGPR strains. The plates were then incubated at 28°C and observed for growth, if any.

Biocompatibility test between microbial strains

Nutrient agar medium was used for checking the compatibility of *Rhizobium* with different combinations of the available microbial strains. Three standard methods of checking biocompatibility *viz*. Well Diffusion method, Cross Streaking, Disc and Swab method were used.

Preparation of modified RL and BL solutions

The microbial cultures were grown in their specific media/ broth. *Rhizobium* was grown in Yeast Mannitol Broth (YEMA, Hi Media, M721), *AzoR* in Jensen's Broth (Hi Media, GM973) and *ECM* in King's B broth (glycerol-10 gpl, K_2HPO_4-1.5 gpl, $MgSO_4$-1.5 gpl, Peptone-20 gpl). Nutrient broth (HiMedia) was used for growing *Bst. (S), Bst.(W)*, *PSB-NB* and *B.sf* All the inoculated plates were incubated for 48 H at 28°C. The 48 H growth cultures were used as primary inoculum (1% of broth) to inoculate the autoclaved and un-autoclaved broth of RL and BL. The biocompatible cultures were inoculated at the rate of 1% each. The inoculated RL and BL solutions were then incubated at 28°C for a period of 48 H. Another set was maintained without incubation- "instant sets". The treatments of different PGPR strains and RL or BL used are given in Table 1 and 2.

Surface sterilization of mung seeds

Seeds were surface sterilized using 0.01% mercuric chloride solution. For this, Mung seeds were dipped in mercuric chloride solution for 3 minutes and then washed 10 times with sterilized distilled water. The seeds were then allowed to dry inside the Laminar Air Flow chamber.

Table 1: Un-autoclaved and autoclaved set of RL used for *in vitro* seed germination assay of mung bean.

Set-A (Un-autoclaved RL) 48 hrs	Set-B (Autoclaved RL) 48 hrs
Un-autoclaved tap water	Un-autoclaved tap water
Autoclaved tap water	Autoclaved tap water
RL	RL
RL+BM	RL+BM
RL+MR	RL+MR
RL+MR+ BM	RL+ MR+BM
RL+Gypsum + MR + BM	RL+Gypsum+MR+BM
RL+Azo R + BM	RL+Azo R+BM

***In vitro* seed germination study**

In the *in vitro* seed germination assay, the study aimed to investigate the impact of modified RL/BL solutions on the germination potential of the certified mung bean seeds (*Vigna radiata)* (Variety bullet) procured from M/S Rajasthan agriculture research institute (RARI), Durgapura, Jaipur. Petri dishes were autoclaved with the circles of Whatman filter paper number 1. The seeds were soaked for four hours in the RL and BL solutions, along with a control set of soaking seeds in tap water.

Estimation of the vigour index

In each of the experiments conducted, the vigour index for each treatment was determined according to the formula from Abdul-Baki and Anderson (1973).

Vigour index = [Mean of root length (cm)+Mean of shoot length (cm)] × Percentage of seed germination

RESULTS AND DISCUSSION

Examining the capability of microbial cultures to utilize RL and BL as the sole source of nutrients

All the microbial cultures procured were evaluated for their ability to utilize autoclaved and un-autoclaved RL and BL. All the cultures could grow very well in the autoclaved RL plates. In the case of un-autoclaved RL plates, apart from the inoculated cultures, the indigenous bacteria of the RL also grew in the plates. However, the inoculated bacteria showed growth along the streaks. The culture growth on solidified RL plates is shown in Fig 1A (autoclaved) and 1B (un-autoclaved). Similarly, the cultures grown on solidified BL plates are shown in Fig 2A (autoclaved) and 2B (un-autoclaved).

Characterization of *Rhizobium* strains

The three *Rhizobium* strains were characterized morphologically and biochemically. The colony

Table 2: Un-autoclaved and autoclaved sets of RL and BL with the given consortiums of PGPR strains used.

Set-C (Un-autoclaved RL/BL)*		Set-D (Autoclaved RL/BL)	
Instant set	48 hrs Incubation	Instant set	48 hrs incubation
Tap water	Tap water	Tap water	Tap water
RL/BL+MR+BM	RL/BL+MR+BM	RL/BL+MR+BM	BL+MR+BM
RL/BL+MR+B.sf	RL/BL+MR+B.sf	RL/BL+MR+B.sf	BL+MR+B.sf
RL/BL+MR+B.st (S)	RL/BL+MR+B.st (S)	RL/BL+MR+B.st (S)	BL+MR+B.st (S)
RL/BL+MR	RL/BL+MR	RL/BL+MR	RL/BL+MR
RL/BL+MR+PSB-NB	RL/BL+MR+PSB-NB	RL/BL+MR+PSB-NB	RL/BL+MR+PSB-NB
RL/BL+MR+ECM	RL/BL+MR+ECM	RL/BL+MR+ECM	RL/BL+MR+ECM
Only RL/BL	Only RL/BL	Only RL/BL	Only RL/BL
BL+MR+B.st (W)	RL/BL+MR+B.st (W)	BL+MR+B.st (W)	BL+MR+B.st (W)

*- RL/BL shows the use of RL or BL with the given combination of PGPR strains *i.e*. both RL and BL were used separately under the autoclaved and un-autoclaved form with the addition of PGPR strains in a similar manner as given in the table.

characteristics on YMA plates and microscopy showed a typical *Rhizobium* growth pattern.

Biochemical characterization of rhizobial strains

All the *Rhizobium* strains showed typical growth on YMA. The colonies were white to cream with exopolysaccharides secretion. The colonies didn't take the colour of congo red dye when grown on YMA plates. All the strains were gram-negative and confirmed by Methyl Red (MR test), Catalase and Bromothymol Blue tests. The negative response was recorded for the Voges Proskauer (VP) test, Starch Hydrolysis and Citrate Utilization tests, which showed the absence of Enterobacteriaceae, common contaminants. All the strains endured temperatures up to 45°C. Chowdhury (2015) and Tyagi *et al.* (2017) reported a similar trend in the biochemical tests.

Biocompatibility studies

In natural conditions, bacteria live in communities and show various kinds of interactions like mutualism, antagonism, synergism, *etc*. Therefore, their biocompatibility is essential for the use of microbial consortiums. The natural ability or capability of microbes living or existing together in harmony reflects the biocompatibility among them. So, it is always recommended that the biocompatibility of the proposed microbial strains be checked before developing a biofertilizer sample. Kumar and Chandra (2008) have also suggested that the compatibility of *Rhizobium* sps. should

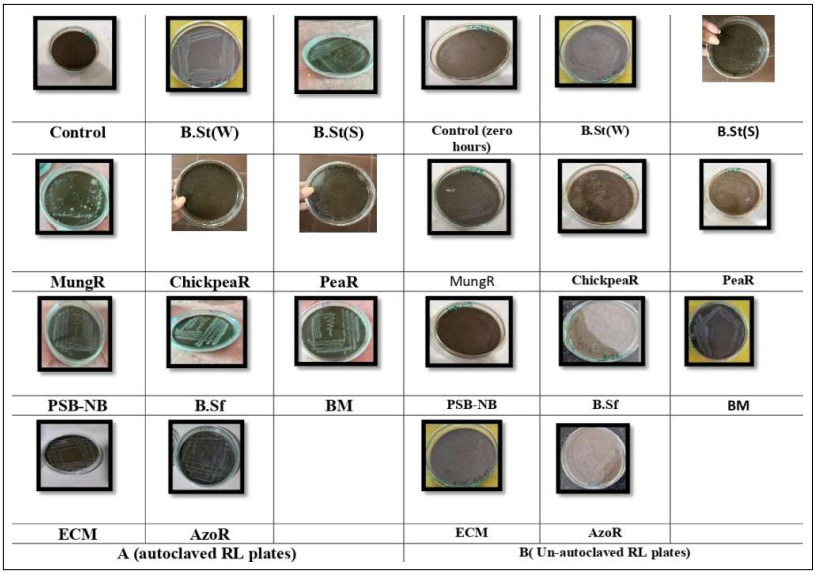


Fig 1: Growth of microbial cultures on autoclaved (A) and un-autoclaved (B) RL plates.



Fig 2: Growth of microbial cultures on autoclaved (A) and un-autoclaved (B) BL plates.

be evaluated before using different consortiums in the field while studying Lentil plants using PSB and PGPRs as biofertilizers. Therefore, the biocompatibility check was performed in the study. The MR was found to be compatible with bacterial strains *viz*. BM, ECM, PSB, Bst.(W) and Bst (S) (Table 3). On the other hand, B.*sf* was found to dominate over *Rhizobium* sps. Whereas, *Rhizobium* sps. dominated over all the other *Bacillus* sps. Overall, MR was found to dominate all the bacterial strains tested except for *B.sf* Based on phosphate solubilization, IAA production and ARDRA profile, *Rhizobium* and *Bacillus* strains have been reported to be compatible with each other (Dhole *et al.,* 2022).

***In vitro* seed germination assay**

The germination assay was carried out to screen the microbes+ RL or BL combination, which showed effective PGP activity. As per the results shown in Table 4, RL+MR was the best in shoot length for both the autoclaved (39.62% over control) and un-autoclaved (9.83% over control) sets. The un-autoclaved RL+MR was the best combination for root length, resulting in an increase of 32.35% in root length than the control. However, in the autoclaved version, maximum root length could be achieved by using a combination of RL+BM+MR+Gypsum. This showed an increase of 30.31% over control. Maximum fresh weight was achieved in the case of un-autoclaved RL+MR (90.72% over control). In the autoclaved set, RL gave the maximum fresh weight (*i.e*. 7.58% higher than the control). Dry weight was obtained with the combination of RL+BM+MR+Gypsum for both the autoclaved and un-autoclaved sets, which was 120% and 141.46% higher than the respective control values (Table 4).

For the autoclaved sets of instant RL, maximum shoot length and fresh weight could be achieved in the RL+MR+PSB treatment and maximum root length (6.66% over control) was obtained in the RL+MR+BM treatment. Maximum dry weight was obtained in the RL+MR+ECM, which was 28.16% higher than the controls (Table 5).

For the un-autoclaved set of instant RL, maximum shoot length was obtained in tap water, whereas maximum root length (530%) and fresh weight (55.55%) were obtained in only RL. Dry weight was maximum (18.18% over control) in the case of only RL and RL+MR+BM.

For the autoclaved set of instant BL, maximum shoot length was obtained in the case of BL+MR+ECM (6.35% more than the control) and maximum root length was found equal in the case of water and BL+MR+ECM. Fresh weight was found to be maximum in the case of water, while dry weight was maximum (1.64% increase over control) in the case of BL+MR (Table 6).

For the un-autoclaved set of instant BL, shoot length (14.35% more than the control), fresh weight (57.44% higher than the control) and dry weight (which was 12.08% more than the control) were found to be the best in BL+MR+B.*st*(W) while maximum root length was achieved in the case of BL+MR+BM which showed an increase of 24.76% over control.

For the autoclaved set of RL-48 hrs, maximum shoot length (*i.e*. 81.12% more than the control) was achieved in the case of RL+MR+PSB while maximum root length (83.25% over control) was found in RL+MR+B.*sf* Both the maximum fresh weight (146.26% higher than the control) and dry weight (98% more than the control) were obtained in only RL (Table 7).

For the un-autoclaved set of RL-48 Hrs, maximum shoot length (8.9% higher than the control) was obtained in the case of RL+MR+BM whereas maximum root length (*i.e.* 51.81% higher than the control) and fresh weight (108% more than the control) were found in only RL. Dry weight (12.67% more than the control) was maximum in the case of RL+MR.

For the autoclaved set of BL-48 hrs, maximum shoot length (67.72% higher) and fresh weight (6% more than the control) were obtained in the case of BL+MR+ECM, whereas maximum root length and dry weight were found in the case of tap water (Table 8).

Table 3: Compatibility among the microbial cultures.

Species	Swab method	Disc method	Cross streak method
Mung *R./B. megaterium*	Positive	Positive	Positive
B. megaterium/mung R.	Positive	Positive	Positive
Mung R./B. safensis	Positive	Positive	Positive
B. safensis/moong R.	Negative	Negative	Positive but *B. safensis* dominating over mung *R*
Mung R/Bacillus sp.(S)	Negative	Negative	Negative
Bacillus sp.(S)/Mung R	Positive	Positive	Positive
Azo R/Mung R (MR)	Positive	-	-
Mung R/Azo R	Negative, MR dominated over AzoR	-	-
ECM/Mung R	Positive	-	-
Mung R/ECM	Negative	-	-
PSB-NB/Mung R	Positive	-	-
Mung R/PSB-NB	Negative as MR dominates over PSB-NB	-	-

Table 4: *In vitro* seed germination assay using Un-autoclaved and autoclaved RL added with different microbial cultures.

Sample details	% of germination	Average shoot length (cm)	Average root length (cm)	Roots hairs and branching	Total FW (g)	Total DW (g)	Vigour index
Un-Autoclaved							
Tap water	100	12.2±1.39	4.76±1.355	RH+++ B++	0.97	0.41	1696
RL	100	9.58±3.88	5.18±1.56	RH++ B++	1.65	0.63	1476
RL+BM	100	9.6±3.50	5.4±2.84	RH++++ B+++	1.65	0.65	1500
RL+MR	100	13.4±2.07	6.3±2.05	RH+++ B+++	1.85	0.79	1970
RL+BM+MR	100	10.9±2.35	5±2.34	RH++++ B++++	1.61	0.70	1590
RL+BM+MR+ Gypsum	100	11±5.21	5.26±3.23	RH+++ B++	1.42	0.99	1626
Un-RL+Azo R+BM	80, 20% fungal contamination	12.25±2.19	6±2.17	RH+++++ B+++++	1.21	0.95	1460
Autoclaved							
Tap water	100	10.6±2.3	3.76±1.21	RH++++ B -	1.45	0.45	1436
RL	100	14.4±1.6	4.56±1.1	RH+++ B+	1.56	0.58	1896
RL+BM	100	10.5±3.8	4.56±1.4	RH++, B+	1.24	0.94	1506
RL+MR	100	14.8±2.1	3.82±1.7	RH++, B+	1.26	0.98	1862
RL+BM+MR	100	10±4.4	3.2±1.3	RH++, B-	1.09	0.59	1320
RL+BM+MR+Gypsum	100	12.4±3.7	4.9±1.7	RH+++, B -	1.44	0.99	1730
RL+Azo R+BM	100	7.5±1.4	4±0.9	RH+B -	1.00	0.90	1150

Leaf pairs-One each in all the germinated seedlings, seedling survival-100% in all the cases tested, All the above values are average±standard deviation of five replicates.

Table 5: *In vitro* seed germination assay (Un-autoclaved and autoclaved set of RL-instant).

Sample details (RL-Instant)	% Germination	% Contamination	Average root length (cm)	Average shoot length (cm)	Branching and root hairs	Total FW (grams)	Total DW (grams)	Vigour index
Un-Autoclaved								
Tap water	100	0	0	3.9±1.38	B+, RH-	0.3607	0.22	460
RL+MR+BM	100	0	3.4±1.24	0.18±0.18	B+, RH-	0.4701	0.26	358
RL+MR+B.*sf*	100	0	3.6±2.41	2.25±1.08	B+, RH-	0.4425	0.17	585
RL+MR+B.*st*	100	0	3.3±2.08	1.7±0.84	B++, RH-	0.4300	0.19	500
RL+ MR	100	0	2.5±0.86	0.99±0.51	B+, RH-	0.3264	0.21	349
RL+MR+PSB	100	0	2.6±1.083	1±0.35	B+, RH+	0.3713	0.19	360
RL+MR+ECM	100	0	2.6±1.63	1.375±0.41	B++, RH-	0.4122	0.18	397
RL	100	0	4.41±1.44	1.6±0.55	B++, RH-	0.5683	0.26	601
Autoclaved								
Tap water	80	20	1.5±0.35	4.33±3.5	B+, RH-	0.68	0.174	466.4
RL+MR+BM	100	0	1.6±1.5	1.7±1.4	B+, RH-	0.71	0.219	330
RL+MR+B.*sf*	80	20	0.6±0.5	1.05±0.7	B+, RH-	0.54	0.202	132
RL+MR+B.*st*	100	0	0.76±0.3	2.7±2.4	B++, RH-	0.78	0.193	346
RL+ MR	100	0	1.1±1.3	1.66±1.0	B+, RH-	0.76	0.207	276
RL+MR+PSB	80	20	0.75±0.2	7.125±4.0	B+, RH+	1.024	0.167	629.6
RL+MR+ECM	100	0	0.62±0.2	2.2±1.9	B++, RH-	0.74	0.223	282
RL	100	0	1.2±0.8	1.7±1.3	B++, RH-	0.85	0.22	290

All values are average±standard deviation of five replicates.

Table 6: *In vitro* seed germination assay (Un-autoclaved and autoclaved set of BL-Instant).

Sample details (BL-Instant)	% Germination	% Seedling survival	Average shoot length (cm)	Average root length (cm)	Branching and root hairs	Total FW (g)	Total DW (g)	Vigour index
Un-Autoclaved								
Tap water	80	100	7.87±5.6	5.37±3.4	B+++, RH+++	0.90	0.182	1059.2
BL+MR+BM	100	100	3±0.6	6.7±3.8	B+, RH+	1.055	0.172	970
BL+MR+B.sf	100	100	8.3±3.6	2±0.6	B +, RH+	0.503	0.094	1030
BL+MR+B.st (S)	20	100	7.25±3.3	1±0	B ++, RH++	0.37	0.12	165
BL+MR	80	100	5.9±3.4	2.25±1.6	B +, RH+	0.628	0.135	652
BL+MR+PSB-NB	100	100	4.5±4	1.4±0.7	B -, RH+	0.712	0.171	590
BL+MR+ECM	80	100	8.37±3.4	1.62±0.4	B -, RH+	0.52	0.056	799.2
BL only	80	100	7.625±0.9	2.25±0.5	B+, RH-	0.8533	0.0911	681.6
BL+MR+B.st (W)	100	100	9±0.2	5.8±0.8	B ++++, RH++++	1.417	0.204	1480
Autoclaved								
Tap water	80	100	7.87±5.6	5.37±3.4	B+++, RH+++	0.90	0.182	1059.2
BL+MR+BM	60	100	3±0.6	2.16±0.8	B-, RH+	0.31	0.08	309.6
BL+MR+B.*sf*	100	100	8.3±3.5	2.6±0.6	B ++, RH++	0.67	0.10	1090
BL+MR+B.st(S)	100	100	7.87±3.7	1.87±0.7	B ++, RH++	0.82	0.14	974
BL+MR	60	100	5.9±3.4	2.3±1.04	B ++, RH++	0.86	0.185	492
BL+MR+PSB-NB	80	100	4.5±4	1.1±0.6	B ++, RH++	0.68	0.16	448
BL+MR+ECM	80	100	8.37±3.4	5.37±1.6	B ++, RH++	0.46	0.07	1099.2
BL only	60	100	7±2.5	2.66±0.4	B+, RH-	0.4334	0.0702	579.6
BL+MR+B.st(W)	80	100	6.13±0.6	0.87±0.2	B +, RH+	0.65	0.12	560

All values are average±standard deviation of five replicates.

Table 7: *In vitro* seed germination assay (Un-autoclaved and autoclaved set of RL-48H).

Sample details (RL-48 hours)	% Germination	% Contamination	Average shoot length (cm)	Average root length (cm)	Roots hairs and branching	Total FW (grams)	Total DW (grams)	Vigour index
Un-Autoclaved								
Tap water	100	0	14.37±2.28	5.5±1.91	RH++.B+	0.752	0.1136	1987
RL+MR+BM	75	25	15.66±0.62	5±1.23	RH++, B+	0.582	0.090	1549.5
RL+MR+B.*sf*	75	25	7.33±4.69	3.166±2.39	RH+, B+	0.353	0.070	786.75
RL+MR+B.*st*(S)	100	0	11.75±1.89	2.62±1.38	RH++, B -	0.705	0.125	1437
RL+MR	100	0	12.75±2.21	5.37±1.70	RH++,B-	0.778	0.128	1812
RL+MR+PSB	100	0	13.37±1.31	5.62±2.78	RH++,B -	0.734	0.100	1899
RL+MR+ECM	100	0	10±3.03	4±2.16	RH+ , B -	0.52	0.097	1400
RL	100	0	11.1±2.18	8.35±4.72	RH+,B+	1.56	0.099	1945
Autoclaved								
Tap water	100	0	8.37±4.15	4±2.48	RH++,B -	0.67	0.05	1237
RL+MR+BM	75	25	8.62±1.11	4.7±1.76	RH++, B+	0.71	0.08	999
RL+MR+B.*sf*	75	25	9.66±2.46	7.33±2.72	RH+, B+	0.64	0.097	1274
RL+MR+B.*st*(S)	100	0	11.125±4.85	3.625±2.98	RH++, B -	0.73	0.055	1474
RL+MR	100	0	13.37±6.21	4.5±2.34	RH++, B -	1.046	0.084	1787
RL+MR+PSB	75	25	15.16±2.1	6.66±2.39	RH++, B -	1.04	0.058	1636.5
RL+MR+ECM	100	0	10±3.03	4±2.16	RH+, B -	0.66	0.084	1400
RL	100	0	12.62±5.01	4.45±1.22	RH+, B++	1.65	0.099	1707

Table 8: *In vitro* seed germination assay (Un-autoclaved and autoclaved set of BL-48 hours).

Sample details (BL-48 hours)	% Germination	% Contamination	Average shoot length (cm)	Average root length (cm)	Branching and root hairs	Total FW (grams)	Total DW (grams)	Vigour index
Un-autoclaved								
Tap water	100	0	13.2±4.56	7.4±2.04	B+++,RH+++	1.052	0.137	2060
BL+MR + BM	100	0	12.8±6.56	5.6±3.27	B+++, RH+++	0.832	0.177	1840
BL+MR + B.*sf*	80	20	6.37±3.21	1.87±1.19	B++, RH++	0.508	0.121	659.2
BL+MR + B.*st*(W)	100	0	8.8±6.16	3.1±2.84	B+++, RH++	0.510	0.149	1190
BL+MR	100	0	11.5±3.89	4.7±1.79	B+++, RH+++	0.701	0.129	1620
BL+MR + PSB	100	0	11.4±4.42	4.5±2.03	B+++, RH+++	0.670	0.127	1590
BL+MR +ECM	100	0	7.1±7.43	2±1.22	B+, RH+	0.828	0.153	910
BL only	100	0	5.8±1.823	1.36±0.61	B+, RH++	0.82	0.13	716
BL+MR + B.*st*(W)	100	0	12.4±5.95	2.9±0.89	B+++, RH++	0.612	0.120	1530
Autoclaved								
Tap water	80	20	7.87±5.64	5.37±3.38	B+++, RH+++	0.90	0.182	1059.2
BL+MR + BM	100	0	11.25±4.78	1.87±0.41	B+++, RH++	0.48	0.099	1312
BL+MR + B.*sf*	100	0	11.25±0.18	3±0.71	B-, RH+	0.307	0.05	1425
BL+MR + B.*st*(W)	20	80	10.4±4.71	2.3±1.35	B+++, RH++	0.79	0.15	254
BL+MR	80	20	8±0.93	3±1.06	B++, RH++	0.3907	0.10	880
BL+MR + PSB	100	0	9±1.06	1.66±0.20	B+++, RH++	0.3920	0.097	1066
BL+MR + ECM	80	20	13.2±3.11	2.2±1.20	B+++, RH++	0.954	0.133	1232
BL only	80	20	7±4.13	3.85±2.79	B+++,RH++	1.27	0.08	315
BL+MR + B.*st*(W)	100	0	10.33±2.61	4.25±2.08	B+++, RH++	0.59	0.14	458

All values are average±standard deviation of five replicates.

For the un-autoclaved set of BL-48 hrs, maximum shoot length, root length and fresh water were obtained in the case of tap water. However, if we compare it with the autoclaved water, then the BL+MR+BM showed the maximum result for root length. Similarly, dry weight (29.19% more than the control) was also the maximum for BL+MR+BM.

Ahmad *et al.* (2012) have reported that the combined application of Rhizobium and Plant Growth Promoting Rhizobacteria (PGPR) improves the growth and productivity of Mung bean (*Vigna radiata* L.) under salt-stressed conditions compared with the un-inoculated control or with the individual inoculation of either the Rhizobium or the Phosphate Solubilizing Bacteria (*Pseudomonas* sps.). In similar lines, mung *Rhizobium* has shown better effects in combination with the PGPR strains used.

Similarly, Korir *et al.* (2017) have also shown the synergistic effect of *Rhizobium* sps. and PGPR, especially the BM, in the bean growth of another leguminous plant, namely *Phaseolus vulgaris* L., *i.e.* common bean. This is in concurrence with our results of getting a better effect of mung Rhizobium when combined with the BM.

As per Kaur and Sharma (2016), out of the various bio-fertilizer treatments on *Vigna radiata* L (mung bean), the net returns of combined inoculation of *Rhizobium* and PGPR were found to be maximum. Although they reported that seed yield increased with the inoculation of different biofertilizers, the combined inoculation of *Rhizobium* and PGPR produced significantly higher seed yield over all the treatments. So, they also reported the synergistic effect of *Rhizobium* and PGPR sps. on mung bean plants.

In one of the studies reported by Yadav *et al.* (2024), the plants grown with individual biofertilizers of *Rhizobium sps*. did not show significant results in the morphological and physical parameters. Still, the consortium of biofertilizers, *i.e.*, *Rhizobium sps., Pseudomonas putida and Frateuria aurantia*, showed observable yield improvements in mung bean plants.

The PGPR strain named *Enterobacter kobei* has shown good response while using the BL. Hayat *et al*. (2010) have also reported that the bacteria belonging to the species *Azospirillum*, *Enterobacter*, *Klebsiella* and *Pseudomonas*, have been shown to attach to the root and efficiently colonize root surfaces in which *Rhizobium* bacteria is already present. Therefore, such PGPRs have the potential to contribute to sustainable plant growth promotion in a synchronized manner if they are compatible with each other.

In the case of microbial consortia, even though they are compatible, their combinations may not always result in higher yields. Many other ecological interactions are going on in the rhizosphere upon consortium application. These interactions may affect the performance of bioinoculants, be it positively or negatively. Inoculants and their consortiums do have an effect on plant growth and yield, but many combinations have to be tried to achieve higher ecological success (Renu *et al.,* 2016).

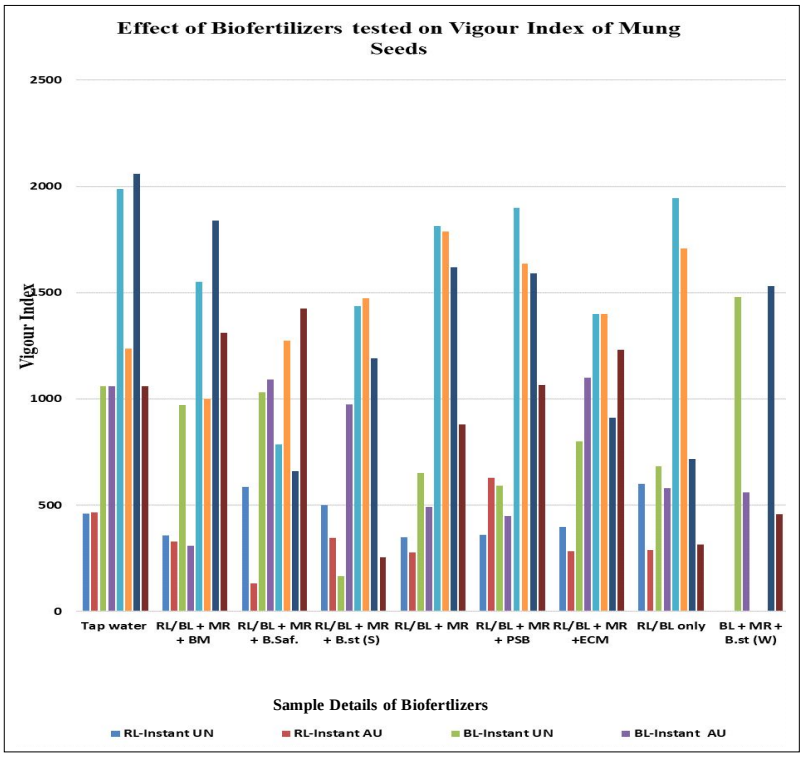


Fig 3: Effect of biofertilizers tested on vigour index of mung seeds.

Khan *et al*. (2026) reported the role of Plant Growth Promoting Rhizobacteria (PGPR) and plant growth regulators in enhancing the growth of chickpea (*Cicer arietinum* L.) and minimizing pesticide induced stress in plants.

Neha *et al.* (2024) demonstrated that co-inoculation of PGPR and *Rhizobium* with *Vigna radiata* significantly enhanced root nodulation, biomass accumulation, nutrient uptake and yield attributes. The mung bean was compared with un-inoculated control and single inoculation treatments. Improved soil health and higher N and P uptake indicated the synergistic role of both microbial inoculants in promoting plant growth and soil fertility.

Thus the findings of the present study are in concurrence of the previous reports indicating a positive effect of using *Rhizobium* in combination with selected PGPR strains. Therefore, an integrated application of potent PGPR strains and *Rhizobium* with handmade paper industrial effluents may serve as an effective and sustainable approach for improving mung bean productivity. Fig 3 shows the overall effect of biofertilizers tested on vigour index of mung seeds.

CONCLUSION

From the systematic detailed study, it can be concluded that the effluents of cow dung based handmade papermaking are capable of supporting the growth of bacterial cultures tested. Out of the two types of effluents (RL and BL), RL was found to be a better option for promoting the plant growth parameters. Nevertheless, the quality of these effluents may be improved by inoculating them with the consortium of biocompatible PGPR strains. Both the types of effluents showed a higher biofertilizer potential in un-autoclaved form than the respective autoclaved forms. Overall, it can be concluded from the germination assay results that RL+ MR is the best combination for both autoclaved and un-autoclaved versions when it was used after 48hours incubation. However, in the case of instant utilization of the modified RL, only RL or water was found to be the best. This might be because the inoculated bacteria could not grow to the sufficient number capable enough to bring out the positive changes. Thus for both RL as well as BL, addition of MR was found to be very effective. Further, the addition of BM with MR showed positive results in both RL as well as BL. This combination was also found best when natural additives like Gypsum was added to modify the effluents.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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