ANSF APPLIED AND NATURAL SCIENCE FOUNDATION

Journal of Applied and Natural Science
16(1), 400 - 409 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Waste-liquors generated during Handmade paper manufacture from cow dung as a potential source of biofertilizer

Satya Narayana
Department of Chemical Engineering, SRM Institute of Science and Technology, Kattankulathur- 603203 (Tamil Nadu), India; Khadi & Village Industries Commission (KVIC), Mumbai-400056, (Maharashtra), India
Kiruthika Sathiasivan
Department of Chemical Engineering, SRM Institute of Science and Technology, Kattankulathur- 603203 (Tamil Nadu), India
Sunita Chauhan*
Kumarappa National Handmade Paper Institute (KNHPI), Ramsinghpura, Sikarpura Road, Sanganer, Jaipur-302029 (Rajasthan), India
Mathur Rajesh*
Department of Chemical Engineering, SRM Institute of Science and Technology, Kattankulathur- 603203 (Tamil Nadu), India

*Corresponding authors. E-mail: knhpibiotechlab@gmail.com; rajeshm@srmist.edu.in

Article Info
https://doi.org/10.31018/jans.v16i1.5209
Received: December 10, 2023
Revised: March 2, 2024
Accepted: March 8, 2024

How to Cite
Narayana S. *et al.* (2023). Waste-liquors generated during Handmade-paper manufacture from cow dung as a potential source of biofertilizer. *Journal of Applied and Natural Science*, 16(1), 400 - 409. https://doi.org/10.31018/jans.v16i1.5209

Abstract
In India, cow dung is widely utilized to create formulations that serve as effective plant growth enhancers with antimicrobial properties. The cow dung-derived handmade paper manufacturing process produces two waste-liquor streams, a Raw Liquor (RL) produced through the dewatering of the cow-dung slurry and a Black Liquor (BL) produced during the soda pulping of dewatered cow dung. The present study explored the potential of these waste streams to be used as plant biofertilizers for germination and cultivation of *Vigna radiata* seeds (mung bean, IPM-02-03 variety). An *in vitro* assay for seed germination efficiency and a pot study for plant growth promotion (PGP) activity were used to assess this potential. The *in vitro* assay demonstrated that nutrient-rich RL, with its 100% seed germination efficiency (better than the 85.7% of tap water) was an effective biofertilizer for seed germination. In contrast, BL yielded poor seed germination efficiency. The pot study showed that water irrigation led to good seed germination, survival, plant rooting and shooting, but it was probably deprived of nutrients for inducing good grain yields. When water was replaced with RL, it was able to replicate the results, but with good grain yields. In contrast, BL produced poorer germination, seed survival and PGP results. The poor biofertilization efficiency of BL was most probably due to the nutrient losses and toxic chemicals produced in the harsh pulping process. The results showed that RL, though considered a waste stream, is sufficiently nutrient-rich to act as an effective biofertilizer for germinating mung bean seeds and promoting plant growth and grain yields.

Keywords: Biofertilizer, Black liquor, Cow dung, Handmade paper, Raw liquor, Waste-liquor

INTRODUCTION

In the Indian economy, livestock is an important subsector of the agriculture sector. It forms an important livelihood activity for most farmers, supporting agriculture in the form of critical inputs, contributing to the health and nutrition of the household, supplementing incomes, offering employment opportunities and being a dependable “bank on hooves” in times of need. It actually acts as a supplementary and complementary enterprise (Ministry of Fisheries, Animal husbandry and Dairying, GoI, 2023). As per the 20th Livestock Census report (2019), India's total livestock population is approximately 536.76 million, with cattle and buffalo accounting for 36.04% and 20.46% of the total population, respectively. It is not surprising to note that livestock plays a significant role in the socio-economic fabric of India, engaging millions of people directly or indirectly in dairy and poultry farming. During 2017-18, the contribution of the livestock sector to the national Gross Domestic Product (GDP)/Gross Value Added (GVA) was 4.10 percent (Kaur, 2017; Phand, 2021).

It is also important to note that even though the livestock population has consistently grown over the decades, it has also resulted in a concomitant increase in the generation of livestock wastes (Fasake and Dashora, 2021). The current annual production of cattle manure in India is reported to be 2600 million tons, with most of it remaining under utilized (Kaur *et al.,* 2017). The amount of manure produced is determined by a variety of factors, including animal weight, breed, animal husbandry practices, the quantity and quality of feed components, weather conditions and health among others (Ravindranath *et al*., 2005; Avcioglu and Turker,2012).

Dung is the ligno-cellulosic rich excreta of bovine animals. Cow dung is mostly used as plant biofertilizer, cleansing agent, mosquito repellent and fuel source in rural India (Gupta *et al.,* 2016). Cow dung has also been reported as a source of natural therapeutic compounds obtained due to its unique microflora. The rich ligno-cellulosic composition of cow dung has also been reported for conversion as biofuel (Mishra *et al.,* 2020).To utilize its untapped potential, researchers in recent decades have proposed a variety of methods to use animal dung for various applications, including green energy (biogas), nutrient (fertilizer) and heavy metal removal from wastewater (Ojedokun and Bello, 2016; Raj *et al.,* 2014, Kaur *et al.,*2017; Kumar,2010).

In India, cow dung holds a special religious significance and is not regarded as a waste. In rural India, dried cow dung cakes are traditionally used as fuel in domestic hearths. The food cooked on cow dung cakes is reported to prevent nutrient loss. Traditionally, cow dung is collected and allowed to undergo natural microbial decomposition before being used directly as a natural and organic plant biofertilizer and soil quality enhancer (Kumar *et al.,*2020). It has also been used in anaerobic digesters to produce biogas, which may be used as a domestic fuel, while the slurry residue can be used as farm manure (Bonten *et al.,* 2014).

Cow dung reportedly comprises ~80% water, ~14.4% rumen undigested ligno-cellulosic fibrous residues and other rumen metabolites and microorganisms. (Behera and Ray, 2021). Approximate composition of cow dung is 40% crude fiber, 20% carbohydrates, 10.5% ash, 7% crude protein; 4% crude fat, depending upon the feed and fodder given to the cow (Kumar *et al.,*2020). The fermentative action of rich and diverse rumen microflora, mainly bacteria (*Bacillus, Lactobacillus* and *Corynebacterium* sp.), fungi (*Aspergillus* and *Trichoderma* sp.), protozoa and yeasts (*Saccharomyces* and *Candida* sp.) on the ligno-cellulosic components decomposes it to produce an array of metabolites (Gupta *et al.,* 2016; Bhatt and Maheswari, 2019). The metabolites can provide nutrients to the plants and act as growth promoters. They also disable plant pathogens by acting as biological control agents. This also facilitates the introduction of a diverse and symbiotic microbial community to the soil, enhancing its overall health and productivity. The introduction and sustenance of these soil microbial communities are known to play a positive role in various ecological processes, including nutrient cycling, organic matter decomposition and disease suppression (Unc and Goss,2004; Durso and Cook, 2014; Zhang *et al., 2018*). However, these microorganisms' specific role and impact are poorly understood due to a lack of scientific research data (Long et al., 2018; Gao *et al.,* 2019; Habibi *et al.,* 2019).

One of the applications of cow dung is handmade paper manufacturing. Handmade papermaking is a traditional craft that has been practiced for centuries in India. In this process, the cellulosic fibrous material of the cow dung is isolated to produce paper. However, this process generates waste liquors, which are likely to contain residual ligno-cellulosic materials, organic metabolites, and other nutrients. Kumarappa National Handmade Paper Institute (KNHPI), which is a DSIR (Department of Scientific and Industrial Research) recognized institute dedicated to research, development and training, operates under the Khadi and Village Industries Commission (KVIC), Ministry of Micro, Small and Medium Enterprises (MSME), Government of India. Its primary focus is the overall development of the Indian handmade paper industry utilizing environmentally friendly technologies. Among KNHPI's successful innovations is the development of processes to utilize cow dung for the manufacture of handmade paper, paint and distemper. Fresh cow dung is mixed with water (1:1 ratio) to prepare a slurry, which is followed by a dewatering step to isolate the relatively dry fiber-rich squeezed cow dung from the slurry, leaving behind the waste RL. This nutrient and microbiologically-rich RL (Raw Liquor) is usually drained and not used further in the process. The RL, similar to cow dung, may find application as a biofertilizer for agriculture.

To make the process of handmade papermaking from cow dung sustainable and economical, KNHPI has utilized BL (Black Liquor) as raw material for manufacturing various consumer products such as soap, shampoo, floor cleaner, liquid soap and natural dyes. BL is the waste effluent generated from the pulping process of cow dung. However, the RL and BL have not been investigated for their ability to act as a plant biofertilizer and growth promoter. The authors hypothesize that RL and BL are likely to have a biofertilizer potential similar to cow dung. The legume seed, mung bean, also known as green gram, is widely cropped and is a good source of human nutrition in India. Therefore, Mung can be the model seed to prove the hypothesis. Mung bean was chosen for the present study because of the favourable climatic conditions for its cultivation in Rajasthan (Reddy, 2022) and its relatively short crop cycle of around three months. Therefore, the present study aimed to investigate the potential of RL and BL as effective biofertilizers, assessing their impact on the overall growth and yield of mung bean plants (*Vigna radiata)*.

MATERIALS AND METHODS

Material

The fresh cow dung used in the experiments was procured from Pinjrapole Gaushala, Sanganer, Jaipur, India, and processed further to generate waste liquors, as described in the next section. The certified mung bean seeds (*Vigna radiata)* of IPM-02-03 variety were procured from Rajasthan State Seeds Corporation Limited, Jaipur, India and used in all the experiments.

Collection and evaluation of waste liquors

The RL and BL were collected from the in-house cow dung-derived handmade paper manufacturing process at KNHPI, Jaipur (Fig.1). The RL was collected from the cow dung dewatering machine. The pulping of the dewatered, fiber rich squeezed cow dung was conducted with 6% sodium hydroxide solution for 3 h at 100^{o} C.The BL was collected as the byproduct of the pulping process. The liquors were analyzed for various parameters of interest, *viz.* macro and micro nutrient content and microbial load as per the standard test protocols in Table 1.

In vitro seed germination assay and pot study

The *in vitro* seed germination assay studies were conducted in petri dishes by placing Whatman filter paper, followed by autoclave sterilisation. Further experiments involved (i) Soaking step and (ii) Watering step. These steps were conducted with different combinations of three solutions (i) tap water (ii) RL and (iii) BL, as tabulated in Table 2 and described below.

The soaking step of seeds was conducted separately in sterile glass beakers by incubating the mung bean seeds for 4 hours in either tap water, RL, or BL solutions. After incubation , 56 seeds were transferred from the beaker to pre-sterilized petri dishes for the germination studies. The petri dishes were incubated in the dark at ambient temperature until the seeds exhibited signs of germination, usually lasting around 22 to 44 h. During this period, the filter paper was prevented from drying with intermittent addition of the respective watering solution.The experiments were designed and conducted in triplicates in 5 sets, termed as Sets G1-G5 (Table 2).

In the pot study, the experiment was conducted using certified mung seeds (*Vigna radiata,* variety IPM-02-03). The study was designed in sextuplicates with five sets of samples, as shown in Table 3. The Set P1 designated as control used tap water for irrigation. In Sets P2 and P4, the tap water was replaced with RL and BL, respectively. In Set P3, the pots were irrigated alternatively with RL and tap water, while Set P5 had alternative BL and water irrigation. During the pot study, soil used for filling the pots was sterilized through the tyndallization process.Thus, the pot study utilized four experimental and one control treatment. So, the total number of sets was four treatments in sextuplicates and one control in sextuplicates, totalling thirty treatments. Each treatment was conducted with 10 seeds per pot. The pot size was 12 inches high and 10 inches wide. The observations from the treatments were recorded at four-day intervals till the harvest on the 92nd day for the growth of the germinating mung seeds. The observations included the root and shoot length (in cm) of the uprooted plants, the pod yield (in number) and the pulse (in grams) from the different sets of plants to assess the effect of RL and BL on the overall growth and yield of the mung bean plants.

RESULTS AND DISCUSSION

Physicochemical analysis of the waste liquors

The nutrient content and total microbial population of RL and BL collected during the cow dung-derived handmade paper manufacturing process are given in Table 1. The RL obtained after the dewatering process showed a low ash content of 1.8% and a relatively high total solid content of 7.97% (w/v), while the nitrogen, phosphorus and potassium (NPK) were 4.1%, 175 mg/L and 461.7 mg/L respectively. The C/N ratio was 0.92 with marginal presence of other micronutrients, except for iron at 10.01 mg/L. In contrast, the BL obtained after the pulping process of the cow dung showed a marginal increase in ash content to 2.0% and a reduction in the total solids to 6.12% (w/v). The NPK values showed a decrease in nitrogen and phosphorus values to 3.4% and 75 mg/L, respectively, while the potassium was observed to be elevated to 630.1 mg/L. The iron values were also considerably reduced to 3.11 mg/L in BL. The results showed that RL and BL possessed a strong presence of nutrients, especially NPK, crucial for plant growth. The RL, particularly with its considerably higher amounts of nitrate/nitrogen, phosphorus and potassium levels, is a good candidate to be used as a biofertilizer. The microbiological analysis of RL and BL showed that they possessed microbial loads of 7.2×10^{8} and 7.8×10^{6} CFU/mL, respectively, where the lower load of BL may be attributed to the pulping process, which could have induced changes in the liquor composition. Table 1 presents the chemical and microbiological analysis of both the waste liquors: RL and BL. The changes in the chemical and microbiological composition of BL compared to RL may be attributed to the alkaline and high-temperature pulping process. The significance of these

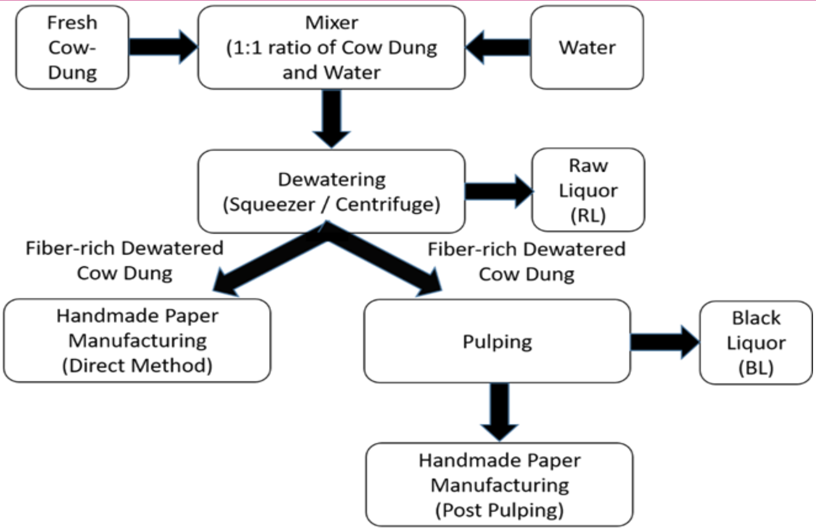

Fig. 1. *Schematic of cow dung derived Handmade paper manufacturing process with generation of Raw Liquor (RL) and Black Liquor (BL) streams*

chemical constituents was assessed by making a comparison with the World Health Organization (WHO) limits for irrigation water. The levels for many of the chemical constituents, especially for Total Solids and NPK are much higher than that of WHO limits. This suggests that RL and BL irrigation has good potential to make essential nutrients bioavailable to plants and also obviate the need for the application of any external inorganic fertilizers .

In vitro seed germination assay

The external factors affecting seed germination are nutrient concentration, nutrient composition, temperature, air, moisture and light (darkness). In this study, the waste liquors, RL and BL were expected to dictate the nutrient concentration and composition. Hence, to determine the biofertilization efficiency of RL and BL, an *in vitro* mung bean seed germination study was conducted in a controlled environment by maintaining

Table 1. Showing physico-chemical and microbiological properties of raw liquor (RL) and black liquor (BL); and the analyical methods used

Parameter	Test Method	Raw Liquor	Black liquor	WHO Limit[(a,b)]
Ash	FCO-1985	1.80%	2.00%	Not applicable#
pH	IS: 3025 (part 11): 1983, Reaff.2017	6.92	8.09	
Total Solids	FCO-1985	7.97% (w/v)	6.12% (w/v)	0-2000mg/l
Nitrate Nitrogen	APHA 23rd Ed. 2017 (4500-NO3-B)	4.10%	3.40%	0-10 mg/l
Phosphorus	APHA 23rd Ed. 2017 (P-4500)	175 mg/L	75 mg/L	0-2 mg/l
Potassium (as K)	CEGTH/STP/C/204	461.70 mg/L	630.1 mg/L	0-2 mg/l
C/N Ratio	FCO-1985	0.92	0.81	10:01
Zinc (as Zn)	CEGTH/STP/C/204	2.92 mg/L	0.80 mg/L	Not applicable#
Calcium (as Ca)	IS: 3025, Part-40: 1991	60 mg/L	564 mg/L	0-20mg/l
Magnesium (as Mg)	IS: 3025, Part-46: 1991	150 mg/L	590.49 mg/L	0-5mg/l
Copper (as Cu)	CEGTH/STP/C/204	0.34 mg/L	0.13 mg/L	2.0mg/l
Lead (as Pb)	CEGTH/STP/C/204	0.10 mg/L	0.06 mg/L	0.01 mg/l
Arsenic (as As)*	CEGTH/STP/C/204	Not detected	Not detected	0.01 mg/l
Cadmium (as Cd)*	CEGTH/STP/C/204	Not detected	Not detected	0.003 mg/l
Mercury (as Hg)*	CEGTH/STP/C/204	Not detected	Not detected	0.006 mg/l
Tin (as Sn)*	CEGTH/STP/C/204	Not detected	Not detected	Not applicable#
Iron (as Fe)	CEGTH/STP/C/204	10.01 mg/L	3.11 mg/L	Not applicable#
Manganese (as Mn)	CEGTH/STP/C/204	4.40 mg/L	1.53 mg/L	0.45mg/l
Total Viable Count (CFU/ml)	IS:5402-2012	7.2×10^8	7.5×10^6	4.0×10^6cells or CFU per 1g bio-fertilizer

*Detection limits (mg/L): As (0.05); Cd (0.01); Hg (0.01) and Sn (0.05), #-No health based guideline value is proposed; Source: a-https://cpcb.nic.in/who-guidelines-for-drinking-water-quality/, b-https://www.fao.org/3/t0234e/T0234E01.html

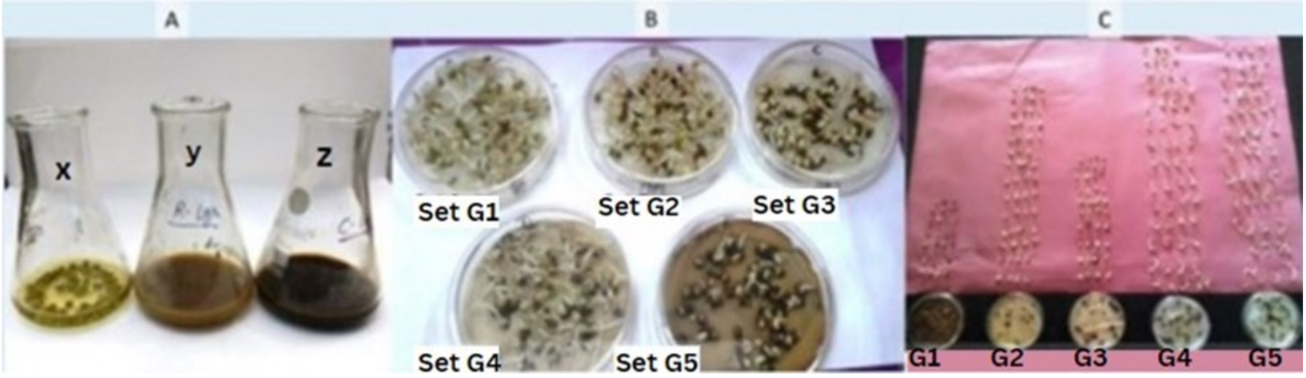

Fig. 2. *In vitro mung bean seed (Vigna radiata) germination assay in petri-dishes with tap water, Raw Liquor (RL) and Black Liquor (BL) solutions. Panel A showing three flasks labeled as X, Y and Z for soaking of the seeds in tap water, RL, and BL respectively; Panel B showing five Petri dishes labeled Sets G1 – G5 with germinating seeds and following the combination scheme of soaking and wetting depicted in Table 2; Panel C showing the stacking of seeds germinated in sets G1 – G5 as described in Table 2 after 44 h incubation*

Table 2. Showing *in vitro* germination (%) assay of mung bean (*Vigna radiata*) and treatments designedwith various combinations of RL, BL and tap water for soaking of the seeds in a beaker before transferring them to petri dishes as Sets (G1 – G5) and watering them *in vitro*

Set	Soaking	Watering	Germination(%)
G1	Tap water	Tap water	85.7
G2	RL	Tap water	100.0
G3	BL	Tap water	71.4
G4	Tap water	RL	100.0
G5	Tap water	BL	50.0

all these external factors constant except for the addition of either RL, BL or water to the germinating seeds as outlined in Table 2. The external factors were kept constant by keeping all the pots in the same area under similar conditions.

The *in vitro* assay involved a primary 4 h. seed soaking step in a beaker followed by their transfer to sterile Petri dishes and a secondary watering step with various solutions (RL, BL and tap water). The filter papers were watered intermittently as and when necessary to prevent them from drying. The assay results conducted in triplicates are summarized in Fig. 2 and Table 2. The results indicated that the control experiment (Set G1), involving tap water soaking and watering, resulted in 85.7% germination rate. Sets G2 and G4, with RL soaking followed by tap water watering and tap water soaking followed by RL watering schemes, respectively, demonstrated a 100% germination rate. Thus, replacing water with RL in the soaking or watering steps produces higher seed germination rates. This is most probably due to nutrients in the RL fertilizing the germination process. The Set G3, with seeds soaked in BL and watered with tap water showed a relatively lower germination rate of 71.43%. The lowest germination percentage, 50% was observed in Set G5, where the seeds were soaked in tap water and watered with BL. The results suggest that replacing tap water in the control experiment with BL in either the soaking or watering steps attenuates the germination efficiency. The *in vitro* assay results have demonstrated the promising biofertilization potential of RL, which needs to be further probed in soil cultivation studies. In contrast, the BL did not appear promising as a biofertilizer. The BL's nutrients were probably bioavailable to the seeds, and the harmful effects (reduction in germination percentage and the reduced root and shoot lengths in the germinated seedlings) were due to other factors induced during the pulping process.

Although it is difficult to pinpoint the reason for the reduced biofertilization efficiency of BL, it may be surmised that this might be due to the high temperature and alkaline pulping conditions. The pulping conditions likely led to evaporative losses and heat-induced thermal and alkaline pH-induced chemical degradation of plant nutrients in the liquor. The degradation can occur due to physical changes, chemical reactions, microbial conversion of the plant residues, and organic fertilizers in the liquor. For example, complex carbohydrates may be hydrolyzed to simpler saccharides, making them bioavailable, but this can also reduce the long-term sustained availability of carbohydrates to soil microorganisms (Paul and Clark,1996). The conditions can degrade the liquor's heat and pH-sensitive biochemicals such as vitamins and enzymes (proteins). The degraded compounds may also affect various biochemical processes in the plants and soil. Hence, minerals themselves may not be susceptible to degradation, but the alterations in the physical and chemical properties of

Table 3. Showing attributes of mung bean (*Vigna radiata*) cultivation of germinated seeds (~10 seeds per pot) for 92 days with a combination of RL, BL and tap water (W) in pots (Observations recorded at four days intervals)

Set	Watering scheme	Germination (%)	Survival of Seedlings (%)	Root Length (cm)	Shoot Length (cm)	Yield (g/ treatment)
P1	W (Control)	86.66 ± 0.94	100.00	6.34 ± 1.58	8.04 ± 1 .17	0.08
P2	RL	83.33 ± 1.25	96.66	10.99 ± 1.97	15.32 ± 3.11	5.87
P3	RL + W	86.66 ± 1.25	96.66	8.93 ± 0.76	16.37 ± 2.91	10.22
P4	BL	6.60 ± 0.47	50.00	3.00 ± 0.33	12.50 ± 1.75	0.00
P5	BL + W	70.00 ± 0.82	64.68	7.35 ± 1.76	13.57 ± 2.46	2.59

the soil may lead to changes in mineral availability and uptake by plants (Marschner, 2012; Higdon and Frei, 2003). Deterioration of soil structure caused by land application of sodium-based BL has also been reported by Xiao *et al.* (2006). A combination of one or more of the above-mentioned factors is likely to have led to the reduced biofertilization efficiency of BL.

Pot study

The *in vitro* assay was only a measure of the ability of RL and BL to facilitate seed germination in petri dishes. A pot study was undertaken to assess their effect in a more realistic soil-based environment. A pot study utilized a soil-filled container to cultivate plants in a near-natural environment. In plant studies, a two-step approach with initial *in vitro* seed germination in a controlled environment followed by its cultivation in a pot environment is usually used. As the germination step influences the crop yield and quality, it was conducted *in vitro* before transferring the seedlings to the pots. This also permits monitoring the early stages of plant development to ensure that only the healthy and uniform seedlings were selected for transfer to the pot study.

However, in the present study, the conventional approach was discarded and experiments were conducted with 5 seeds placed directly in the pots and irrigated with RL, BL and tap water. The experiments were conducted in 30 cms high and 25 cms wide pots, keeping all factors constant except for the irrigation scheme involving RL, BL or tap water, as defined in Table 3.The crop growth progress was monitored at four-day intervals, and the crop was harvested at the end of 92 days. The results of the germination efficiency and PGP are mentioned in Table 3. Fig. 3 shows pictures of the plants during various stages of the pot study. Fig. 4 shows the root and shoot lengths of the potted plants at harvest.

The control set, Set P1, irrigated with tap water, demonstrated an impressive 86.66% germination rate and 100% seedling survival, serving as the benchmark for other sets. Set P2, irrigated with only RL, exhibited germination and seedling survival rates of 83.33% and 96.66%, respectively, comparable to Set P1. In Set P3, where RL and tap water irrigation were alternated, germination reached 86.66%, equivalent to Set P1, while seedling survival remained at 96.66%. This suggests that intermittent tap water irrigation did not compromise the effectiveness of RL as a biofertilizer. However, Set P4, irrigated with BL, displayed a meagre 6.66% germination and 50% seedling survival rate, unequivocally indicating BL's poor biofertilization performance. This trend was also reflected in Set P5, involving alternating BL and tap water irrigation steps. The introduction of tap water seemed to mitigate some of BL's adverse effects, resulting in 70% germination and 64.68% seedling survival rates. In conclusion, these results strongly suggest that RL was an effective biofertilizer, while BL demonstrated poor biofertilization efficiency.

The PGP parameters monitored by recording the root length, shoot length and grain yield at harvest is illustrated in Fig. 3 and Table 3. The results showed that tap water irrigation in Set P1, though producing good germination and seed survival rates, failed the PGP activity test with poor root length (6.34 cm), shoot length (8.04 cm) and grain yield (0.08g) values. Set P2’s excellent germination and seed survival rates were also reflected in the PGP activity results, yielding root length, shoot length and grain yield of 10.99 cm, 15.32 cm and 5.87 g, respectively. When alternate, RL and tap water were employed as designed in Set P3; these values were observed to be 8.93 cm, 16.37 cm and 10.22 g, respectively. These results showed that intermittent introduction of tap water along with RL appeared to produce near equivalent root and shoot lengths, with enhanced grain yields. The PGP activity also reflected the poor germination resulting in Set P4. The BL irrigation produced only 3.00 cm root length, 12.50 cm shoot length and nil grain yield, reinforcing the earlier observation that BL was a poor biofertilizer. The results of SetP5 produced 7.35 cm root length, 13.57 cm shoot length and 2.59 g grain yield. Hence, the deleterious effect of BL appears to be alleviated by replacing it with tap water. An interesting point to note here was that the PGP attributes of BL + Water in Set P5 were better than the absolute control Set P1.

Comparing the overall yields, Set P2 and Set P3 led to 7237% and 12675% increase in yield. Combining these results showed that RL is a good biofertilizer, with its nutrients being bioavailable to the mung seed for germination, survival, and PGP activity. In contrast, BL appeared to be a mung crop growth inhibitor when

Fig. 3. *Pot experiments showing mung bean (Vigna radiata) seeds germination and seedling survival after 43, 60 and 75 DAS (Days After Sowing) with different treatments designated as Sets P1-P5 with a combination of tap water, RL and BL as described in Table 3 and Pot Study section*

used as such, but it supported the plant growth to some extent if used in alternation with water for the irrigation of plants. Therefore, BL appeared to be useful only in its diluted form.

Cow dung, known for its elevated lignocellulosic and nutrient levels, has diverse applications in rural economies, particularly in countries like India. A diverse range of plant growth-promoting bacteria is harbored within cow dung, exhibiting various activities such as cellulase, protease, urease, lipase, and amylase production. Dung is recognized as a crucial element in most biodynamic preparations, acting as a reservoir of beneficial microorganisms.Biodynamic products encompass essential macro and micronutrients, amino acids, and substances that foster growth, including indole acetic acid (IAA), gibberellins, and beneficial microorganisms (Dhiman *et al*., 2019).Behera *et al.* (2021) have also reported bioprospecting of cow dung microflora for sustainable agricultural, biotechnological and environmental applications. Besides its role as a biofertilizer, cow dung has also proven to be a valuable raw material for paper production (Kumar *et al.,* 2020; Yang *et al.,* 2023; Yang *et al.,* 2023a; Fasake and Dashora*,* 2020). However, the papermaking process generates waste-liquor streams with limited applications. This study was initiated with the idea that the nutrient-rich liquid, once separated from its lignocellulosic content, could serve as an effective fertilizer comparable to cow dung. The KNHPI explored two handmade paper manufacturing methods using cow dung. In the first approach, cow dung is squeezed to separate the lignocellulosic-rich solids from the liquid. The solid part is utilized for handmade paper manufacture, while the nutrient-rich liquid fraction, referred to as RL, is considered on par with cow dung as a fertilizer. In the second method, cow dung undergoes dilute alkali treatment at high temperatures and atmospheric pressure to enhance lignocellulosic fraction recovery. The resulting liquor is known as BL. Although the second method yields good paper quality, the pulping conditions used (three hours boiling with 6% sodium hydroxide on oven-dried basis of the cow dung) might be harsh for inherent microflora and, therefore, may compromise the BL's efficacy as a biofertilizer compared to RL.

Fasake and Deshora (2020) have reported the characteristics and morphology of natural cow dung-derived polymers with potential industrial applications, including bio-based filler during handmade paper manufacture. They also reported that the waste liquors have abundant nutrients, including nitrogen, phosphorus and potassium, thus making it a good candidate for fertilizer use. Mukhuba *et al.* (2018) reported that fresh cow dung diluted with water possesses 1.6 ppm ammonium, 0.9 ppm potassium and 0.43 ppm phosphorus content. In the present study, physicochemical and microbiological analysis of the RL and BL demonstrated that these liquors have more than adequate nutrient amounts, especially NPK, and good microbial loads to act as plant fertilizers. The present analysis also established that the mechanical and chemical processing did not completely degrade the nutrient content and transmitted it to the liquid fractions, RL and BL, respectively. Contrary to the findings of Bhatt and Maheshwari (2019), the results regarding physico-chemical properties showed differences, with higher values for pH and NPK, except for electrical conductivity (EC).

Similar observations regarding the PGP characteristics of bacteria from cow dung were reported by Ram *et al.* (2020) and Aiysha *et al.* (2019). In the contemporary agricultural landscape, the commercial marketing emphasises the quality rather than the quantity of crops. This shift in focus is evident in the rising demand for crops cultivated with organic fertilizers, which are currently recognized as standard agricultural best practices (Ahmad *et al.,* 2019). Moreover, the simultaneous use of organic (such as compost from cattle manure) and inorganic (NPK) fertilizers is reported to result in an elevation of soil organic carbon (SOC) and total nitrogen. This was also found to enhance the bacterial community responsible for decomposing complex organic substances and facilitating the transformations of soil carbon, nitrogen, and phosphorus (Li *et al.,* 2019).

During the second step of the present study, *i.e. in vitro* seed germination assay, when compared to the control, the RL enhanced the percentage of mung bean seed germination, while the BL appeared to degrade it. In the third step, the mung bean productivity conducted in pot study with different combinations of tap water, RL and BL irrigation again showed that RL produced superior seed germination, seedling survival, root length, shoot length and grain yields when compared to the control. In comparison, the plant growth-promoting activity of BL was very poor.

The superior results with RL were most probably because it is merely a diluted extract of cow dung, while BL is an alkaline extract of mild-alkali and heat-treated cowdung. The alkaline pulping process is also likely to induce reactions that produce chemical compounds. The reduced seed germination and plant productivity with BL can be attributed to its alkalinity and the likely loss of plant nutrients due to the thermal degradation of one or the other plant nutrients during the boiling process.

In another study by Bello *et al.*(2023), the experiment revealed that the combined utilization of cow-dung manures, mulching and NPK has a noteworthy impact on the production of *Celosia argentea* plant compared to the control group.This discovery highlighted that intensifying *Celosia argentea* production through the integrated use of NPK fertilizer, cow-dung manure and mulching could be a crucial approach for sustainable production. The research findings of Kumar *et al.* (2022) re-

Fig. 4. *Pot study harvested plants showing plant growth promotion parameters, root length, shoot length and grain yield in response to different treatments designated as sets P1- P5 with a combination of RL, BL and tap water as mentioned in Table 3*

vealed that the lasting impact of applying biochar and cow dung manure significantly influenced the subsequent yield of green gram crops in acid sandy loam soils. This influence was attributed to the liming effect, which enhanced soil properties and nutrient accessibility. Particularly noteworthy was the positive outcome observed when using a combination of coconut shell biochar (8 tons per hectare) and cow dung manure (10 tons per hectare) along with the recommended NPK fertilizers.

The present study, meticulously designed *in vitro* conditions involving a four-hour immersion of mung seeds in RL, BL, and tap water, yielded significant results. Set P1, using only tap water, achieved an 85.7% germination rate. Set P2 exceeded expectations, displaying root lengths, shoot lengths, and grain yields of 10.99 cm, 15.32 cm, and 5.87 g. Set P3, with alternative solutions (RL and tapwater), showed increased values of 8.93 cm, 16.37 cm, and 10.22 g. Compared overall, Set P2 and Set P3 exhibited a 7237% and 12675% boost in yield, respectively, emphasizing the substantial impact of RL on the growth and yield of mung plants.

Conclusion

The present study focused on testing the potential of cow dung liquors obtained after removing the fiber-rich ligno-cellulosic components as biofertilizers. The liquor produced from cow dung during the handmade paper manufacturing process (referred as RL) was a valuable source of NPK, other mineral nutrients, and beneficial microorganisms. Applying RL as a biofertilizer demonstrated excellent promotion of the growth and yield of mung bean seeds. On the other hand, the liquor obtained after the pulping process (referred to as BL) was not an effective biofertilizer, likely due to factors such as alkalinity, potential presence of toxic chemicals and a relatively low microbial load besides a probable thermal degradation of plant nutrients. However, diluted BL showed some effectiveness. Therefore, this study suggests that RL could serve as a beneficial source of biofertilizers to replace the use of synthetic fertilizers to a partial, if not complete extent. Further works, including cost-to-benefit and life-cycle analyses related to RL's production cost, the extent of synthetic fertilizer replacement, and shelf-life, are essential for translating this technology to practical field applications and commercializing it. This approach may contribute to the sustainable management of cow dung waste and support the development of a circular economy in society.

ACKNOWLEDGEMENTS

The financial support provided by the Directorate of Science & Technology, Khadi & Village Industries Commission (KVIC), Mumbai, is thankfully acknowledged. Authors are thankful to the Director, KNHPI/SOJ, KVIC, Jaipur, Dr. Rahul Misra, for allowing them to conduct this study at KNHPI. The help of Dr. Poonam Singh, Principal Scientist and Associate Professor, Division of Plant-Microbe Interaction, National Botanical Research Institute (NBRI), Lucknow, for preparing the draft is thankfully acknowledged. We are thankful to Dr.M. Krishna Mohan, Head, R&D, Birla Institute of Scientific Research (BISR), Jaipur, for his help during the initial conceptualization of the study.

Conflict of interests

The authors declare that they have no conflict of interest.

REFERENCES

1. Ahmad, A. T., Bello, I. U., Jibril, S. M., Kolawole, O. S., Ogah, J. J. & Daniel, S. (2019). Comparative evaluation of the effects of organic and inorganic fertilizers on the vegetative growth of spleen amaranth (*Amaranthus dubius* L). *Journal of Applied Sciences and Environmental Management*, *23*(2), 359. https://doi.org/10.4314/jasem.v23i2.24
2. Annual Report (2022-2023). Department of Animal Husbandry and Dairying, Ministry of Fisheries, Snimal Husbandry and Dairying, Govt. of India.
3. Arunkumar, B. R., & Thippeshappa, G. N. (2022). Carryover Effect of Biochar and Cow Dung Manure on the Productivity of Green Gram (Vigna radiata L.) in Typic haplustalf. *LEGUME RESEARCH - AN INTERNATIONAL JOURNAL*, *Of.* https://doi.org/10.18805/LR-4755
4. Arunkumar, B. R. & Thippeshappa, G. N. (2023). *Carryover effect of biochar and cow dung manure on the productivity of green gram (Vigna radiata L.) in Typic haplustalf.*
5. Ashwin Kumar, A. (2010). A study on renewable energy resources in India. *2010 International Conference on Environmental Engineering and Applications*, 49–53. https://doi.org/10.1109/ICEEA.2010.5596088
6. Avcioğlu, A. O. & Türker, U. (2012). Status and potential of biogas energy from animal wastes in Turkey. *Renewable and Sustainable Energy Reviews*, *16*(3), 1557–1561. https://doi.org/10.1016/j.rser.2011.11.006.
7. Behera, S. S. & Ray, R. C. (2021). Bioprospecting of cowdung microflora for sustainable agricultural, biotechnological and environmental applications. *Current Research in Microbial Sciences*, *2*, 100018. https://doi.org/10.1016/j.crmicr.2020.100018.
8. Bello, W. B., Olla, N. O., Ogunjinmi, S. O., Olaniyan, M. I., Oyaniyi, T. O. & Olaifa, G. O. (2023). *Effects of Cow Dung, NPK Fertilizer and Mulching on Zn, Pb Dynamics and Yield of Celosia Argentea*. https://doi.org/10.5281/ZENODO.8216541.
9. Bhatt, K. & Maheshwari, D. K. (2019). Decoding multifarious role of cow dung bacteria in mobilization of zinc fractions along with growth promotion of C. annuum L. *Scientific Reports*, *9*(1), 14232. https://doi.org/10.1038/s41598-019-50788-8
10. Bonten, L. T. C., Zwart, K. B., Rietra, R. P. J. J., Postma, R., de Haas, M. J. G. & Nysingh, S. L. (2014). *Bio-slurry*

as fertilizer : is bio-slurry from household digesters a better fertilizer than manure? : a literature review. (Alterra-report; No. 2519). Alterra. https://edepot.wur.nl/307735

11. Dhiman, S., Dubey, R. C., Maheshwari, D. K. & Kumar, S. (2019). Sulfur-oxidizing buffalo dung bacteria enhance growth and yield of *Foeniculum vulgare* Mill. *Canadian Journal of Microbiology*, *65*(5), 377–386. https://doi.org/10.1139/cjm-2018-0476.
12. Durso, L. M. & Cook, K. L. (2014). Impacts of antibiotic use in agriculture: What are the benefits and risks? *Current Opinion in Microbiology*, *19*, 37–44. https://doi.org/10.1016/j.mib.2014.05.019.
13. Fasake, V. & Dashora, K. (2020). Characterization and Morphology of Natural Dung Polymer for Potential Industrial Application as Bio-Based Fillers. *Polymers*, *12*(12), 3030. https://doi.org/10.3390/polym12123030.
14. Fasake, V. & Dashora, K. (2021). A sustainable potential source of ruminant animal waste material (dung fiber) for various industrial applications: A review. *Bioresource Technology Reports*, *15*, 100693.
15. Gao, J., Luo, Y., Wei, Y., Huang, Y., Zhang, H., He, W., Sheng, H. & An, L. (2019). Screening of plant growth promoting bacteria (PGPB) from rhizosphere and bulk soil of *Caragana microphylla* in different habitats and their effects on the growth of *Arabidopsis* seedlings. *Biotechnology & Biotechnological Equipment*, *33*(1), 921–930. https://doi.org/10.1080/13102818.2019.1629841.
16. Gupta, K. K., Aneja, K. R. & Rana, D. (2016). Current status of cow dung as a bioresource for sustainable development. *Bioresources and Bioprocessing*, *3*(1), 28. https://doi.org/10.1186/s40643-016-0105-9.
17. Habibi, S., Djedidi, S., Ohkama-Ohtsu, N., Sarhadi, W. A., Kojima, K., Rallos, R. V., Ramirez, M. D. A., Yamaya, H., Sekimoto, H. & Yokoyama, T. (2019). Isolation and Screening of Indigenous Plant Growth-promoting Rhizobacteria from Different Rice Cultivars in Afghanistan Soils. *Microbes and Environments*, *34*(4), 347–355. https://doi.org/10.1264/jsme2.ME18168.
18. Higdon, J. V., & Frei, B. (2003). Tea Catechins and Polyphenols: Health Effects, Metabolism, and Antioxidant Functions. *Critical Reviews in Food Science and Nutrition*, *43*(1), 89–143. https://doi.org/10.1080/104086 903908 26 464.
19. Kaur, G., Brar, Y. & Kothari, D. P. (2017). Potential of Livestock Generated Biomass: Untapped Energy Source in India. *Energies*, *10*(7), 847. https://doi.org/10.3390/en10070847.
20. Kumar, G., Tiwari, P. K., Atbhaiya, Y., Upadhaya, V. R., & Yadav, D. K. (2020). Beneficial Use of Vedic Indian Cow Dung. *International Journal of Advances in Agricultural Science and Technology*, *7*(10), 6–10. https://doi.org/10.47856/IJAAST.2020.v07i10.002.
21. Li, F., Liang, X., Niyungeko, C., Sun, T., Liu, F., & Arai, Y. (2019). Effects of biochar amendments on soil phosphorus transformation in agricultural soils. In *Advances in Agronomy* (Vol. 158, pp. 131–172). Elsevier. https://doi.org/10.1016/bs.agron.2019.07.002.
22. Long, C. M., Muenich, R. L., Kalcic, M. M. & Scavia, D. (2018). Use of manure nutrients from concentrated animal feeding operations. *Journal of Great Lakes Research*, *44* (2), 245–252. https://doi.org/10.1016/j.jglr.2018.01.006.
23. *Marschner's Mineral Nutrition of Higher Plants*. (2012). Elsevier. https://doi.org/10.1016/C2009-0-63043-9.
24. Mishra, O., Pathak, R., Parmar, M. S. & Doneria, R. (2020). Cow dung an undeciphered boon: An overview. *The Pharma Innovation*, *9*(11S), 84–89. https://doi.org/10.22271/tpi.2020.v9.i11Sb.5370.
25. Mukhuba, M., Roopnarain, A., Adeleke, R., Moeletsi, M. & Makofane, R. (2018). Comparative assessment of bio-fertiliser quality of cow dung and anaerobic digestion effluent. *Cogent Food & Agriculture*, *4*(1), 1435019. https://doi.org/10.1080/23311932.2018.1435019.
26. Ojedokun, A. T., & Bello, O. S. (2016). Sequestering heavy metals from wastewater using cow dung. *Water Resources and Industry*, *13*, 7–13. https://doi.org/10.1016/j.wri.2016.02.002.
27. Paul, E. A. & Clark, F. E. (1996). Soil microbiology and biochemistry Academic Press. *New York, USA*.
28. Phand,S., Jaya,G. & Gummagolmath, K.C.(2021). Livestock extension services: time to think beyond treatment and breed improvement. ed. Lakshmi Murthy, National Institute of Agricultural Extension Management (MANAGE), Hyderabad. Manage Knowledge Series no.2/2021. Vol.2, August'2021
29. Raj, D., Jhariya, M. & Toppo, P. (2014). Cow Dung For Ecofriendly And Sustainable Productive Farming. *International Journal Of Scientific Research*, *3*, 201–202.
30. Ram, R. A., Kumar, G., Maurya, S. K., Rajan, S. & Ahmad, I. (2020). Plant growth promoting, biochemical and antifungal properties of microbes isolated from cow dung. *Progressive Horticulture*, *52*(2), 166–172. https://doi.org/10.5958/2249-5258.2020.00025.
31. Ravindranath, N. H., Somashekar, H. I., Nagaraja, M. S., Sudha, P., Sangeetha, G., Bhattacharya, S. C., & Abdul Salam, P. (2005). Assessment of sustainable non-plantation biomass resources potential for energy in India. *Biomass and Bioenergy*, *29*(3), 178–190. https://doi.org/10.1016/j.biombioe.2005.03.005.
32. Unc, A. & Goss, M. J. (2004). Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, *25*(1), 1–18. https://doi.org/10.1016/j.apsoil.2003.08.007.
33. Xiao,C.; Fauci, M. ; Bezdicek,D.F. and Pan, W.L. (2006) Soil microbial responses to potassium based black liquor from straw pulping. Soil Sci Soc. Am. J. 70(1) : 72-77. doi: https://doi.org/10.2136/sssaj2004.0339.
34. Yang, X., Li, L., Zhao, W., Tian, Y., Zheng, R., Deng, S. & Mu, Y. (2023). The Influence of Potassium Hydroxide Concentration and Temperature on Pulp Characteristics and Cow Dung-Based Paper Performance. *Journal of Natural Fibers*, *20*(1), 2164546. https://doi.org/10.1080/15440478.2022.2164546.
35. Yang, X., Li, L., Zhao, W., Wang, M., Yang, W., Tian, Y., Zheng, R., Deng, S., Mu, Y. & Zhu, X. (2023). Characteristics and Functional Application of Cellulose Fibers Extracted from Cow Dung Wastes. *Materials*, *16*(2), 648. https://doi.org/10.3390/ma16020648.
36. Zhang, Y., Hao, X., Alexander, T., Thomas, B. W., Shi, X. & Lupwayi, N. (2018). Long-term and legacy effects of manure application on soil microbial community composition. *Biology and Fertility of Soils*, *54*, 269–283. https://doi.org/10.1007/s00374-017-1257-2.