PLANT SCIENCE TODAY
Vol 12(2): 1-11
https://doi.org/10.14719/pst.8087

eISSN 2348-1900

RESEARCH ARTICLE

Effect of PGPR-enriched biofertilizers from handmade paper industry effluents on green chilli (*Capsicum frutescens*) cultivation

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Received: 05 March 2025; Accepted: 07 May 2025; Available online: Version 1.0: 24 May 2025; Version 2.0 : 09 June 2025

Cite this article: Satya N, Sunita C, Rahul M, Mathur R. Effect of PGPR-enriched biofertilizers from handmade paper industry effluents on green chilli (*Capsicum frutescens*) cultivation. Plant Science Today. 2025; 12(2): 1-11. https://doi.org/10.14719/pst.8087

Abstract

The handmade paper industry utilizing non-woody raw materials such as cow-dung produces an organic-matter rich effluent which is relatively less harmful to environment. In this study, a novel biofertilizer formulated by enriching this effluent with selected Plant Growth Promoting Rhizobacteria (PGPR) was tested for its biofertilizing potential against the NU1919 variety of green chilli (*Capsicum frutescens*). The study was conducted using four treatments in triplicates with the Randomized Block Design. Parameters related to plant growthpromotion-including number of fruits per plant, average fruit length, fruit yield per plant, average plant height and number of branches-were recorded. The best performance was observed in Set D, which used a consortium of *Azotobacter* sp. and *Enterobacter* sp. along with unautoclaved effluent (raw liquor, RL). Similarly, average girth and average fruit weight (fresh and dry weight) were also recorded to bemaximum in the red chillies harvested from this Set D. The maximum shoot length was found to be in Set D, whereas the maximum root length was observed in Set A which used the RL without any additional bacterial cultures. Vitamin A was found to be maximum in the case of Set A, whereas Vitamin C was found to be almost equal in two Sets (Set A, 167.79 mg/100 g and Set D, 167.30 mg/100 g). The experimental Set C with *Bacillus* and *Azotobacter* showed maximum capsaicinoid content (67793.18 SHU). This study highlights the potential of PGPR-enriched biofertilizers, formulated using effluent from the handmade paper industry, for enhancing chilli cultivation.

Keywords: biofertilizer; chilli; effluents; handmade paper; PGPR

Introduction

The burgeoning human population coupled with intensified industrial and agricultural activities, has significantly increased the demand for clean freshwater. This water is sourced from groundwater (bore-wells), still-water bodies (lakes, ponds, tanks) and flowing sources such as brooks and rivers. However, increased consumption of these water sources along with climatic changes has led to water scarcity in many highly populated regions of the world. Apart from this, pollution of these water sources poses another challenge for sustaining these sources of clean water.

One of the major reasons for pollution of these water sources is wastewater, an undesirable by-product discharged from commercial, industrial, municipal and residential sources. It is a dilute mixture of water contaminated with small amounts (usually <1 %) of hazardous components which may include microorganisms, organic and inorganic materials. Runoffs with its contaminants find their way to brooks, rivulets, ponds, tanks, lakes and may eventually reach estuaries and oceans. Hence, it is necessary to treat these pollutants with wastewater treatment plants (WWTPs) at the source before discharge into the environment (1).

The objective of the WWTP is to enhance effluent quality by reducing the pollutant levels to tolerable levels while considering the techno-economic efficiency of the process (2). However, efficiency of these plants may be affected by various factors such as plant capacity, location, availability of resources involving manpower, energy, additional freshwater, treatment chemicals, nature of process technology used, environmental effluent discharge regulations, etc. As a result, WWTPs often require complex process control and intensification strategies to function effectively (3).

The pulp and paper industry are one of the major chemical industries of the world consuming large amounts of wood and water resources. It also produces large amounts of dark coloured wastewater bearing ligno-cellulosic residues and highly recalcitrant organic and inorganic chemicals. The treatment of this wastewater requires complex steps and is expensive before it can be released into the aquatic environment. However, most of the energy content in this waste stream remains unutilized. Also, the water is rarely recycled due to its dark coloured and contaminated nature.

In contrast to the conventional industrial process, the handmade paper production industry utilizing non-woody raw materials requires lesser chemicals and produces relatively less complex wastewater. However, the non-woody raw materials such as cow-dung also involve ligno-cellulosic content producing dark coloured effluents post wastewater treatment (4). Therefore, in these non-conventional processes also, there is an unmet need to reduce waste generation and manage effluents. Recent studies have explored the biofertilization potential of effluents from handmade paper manufacturing processes (5), aiming to mitigate pollution, generate supplementary revenue and promote sustainable agriculture.

Capsicum frutescens a wild chilli pepper species from the Solanaceae family is closely related to Capsicum Chinese, both native to Central and South America. These varieties may have either annual or short-lived perennial life cycles (6). The white flowers with either green-white or greenish-yellow corolla are reported to be either insect- or self-pollinated. The berries on the bushes are reported to be usually grow upright and possess ellipsoid-conical to lanceoloid shape (7). The pungent pepper is usually small in size, measuring only around 1-2 cm in length and 0.3-0.7 cm in diameter. The *C. frutescens* fruit initially pale yellow in colour, upon maturation may take on different colours with the vivid red one being most popular in the markets (8). It is one of the most used condiments around the world, second only to table salt; because the spicy taste attracts the attention of millions of people from different cultures, even if its consumption generates a burning sensation in the mouth and throat. It also bears many therapeutic properties due to the medicinally important constituent, capsaicin (9). Chilli peppers can be dehydrated and marketed as flakes containing the seeds and powder. Chillies are rich in Vitamin A, C and E (10). This is expected to preserve the organoleptic characteristics and energy values of the chilli. The lifespan of the chilli plant ranges from 1.5 to 15 years depending on the species and climatic conditions (11). The different parts of a chilli pepper are labelled in Fig. 1.

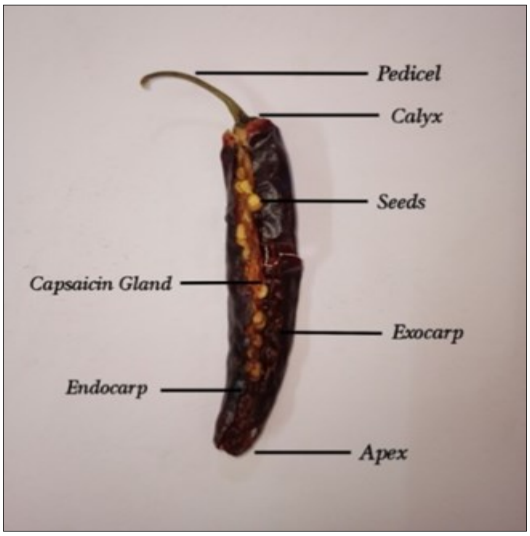

Fig. 1. Parts of a chilli pepper.

Chilli peppers can grow in diverse agro-climatic conditions but thrive best in warm (20-30 °C), humid subtropical climates. In India, it is usually cultivated as a Rabi crop from September to March, which was also utilized in this study conducted in 2023-24. It is referred to as “Green Chillies” in India and is considered as a medicinal herb and used in traditional Indian medical system to treat coughs, toothaches, sore throats, parasitic infections, rheumatism, heal wounds, etc. It is also reported to possess antibacterial and anticancer properties (12). It is also considered to be rich in nutrients and bioactive chemicals, especially alkaloids, flavonoids, phenolics, essential oils, tannins, steroids and capsaicin. Table 1 summarizes the nutritional composition of green chilli (13). Their medicinal value, commercial significance and cultivation ease make them ideal for evaluating the biofertilization potential of effluents from handmade paper industries.

Plant Growth Promoting Rhizobacteria (PGPR) are symbiotic microorganisms that inhabit the roots of plants and indirectly promote plant growth and soil fertility. The symbiotic action results in many activities, such as making bioavailable atmospheric nitrogen and soil-bound phosphates, water and minerals, curbing plant pathogen activity as well, stimulating plant metabolism to produce hormones, etc. Hence, PGPR have found an important role in developing sustainable agriculture practices to mitigate the use of synthetic fertilizers and pesticides, promote plant health and growth as well as enhance soil quality.

This study investigates the potential of enhancing the biofertilization capacity of waste liquor sourced from the handmade paper industry by supplementing it with various PGPR strains, both isolated at Kumarappa National Handmade Paper Institute (KNHPI) and obtained from microbial culture collections. The enriched biofertilizer formulations were then evaluated for plant growth-promoting effects using *Capsicum frutescens* (green chilli) as a model crop.

Materials and Methods

Procurement of standard microbial strains of PGPR

Bacillus megaterium (NAIMCC-B-02287, BM) and *Enterobacterkobei* (NAIMCC-B-02167, ECM) were procured from National Agriculturally Important Microbial Culture Collection (NAIMCC), Indian Council for Agricultural Research - National Bureau of Agriculturally Important Microorganisms

Table 1. Nutritional contents of green chilli (per 100 g of edible portion) (13)

Nutrients	Per 100 g of edible portion of *Capsicum frutescens*
Carbohydrates (g)	9.46
Protein (g)	2.00
Fat (g)	0.20
Energy (Kcal)	40.00
Iron (mg)	1.20
Calcium (mg)	18.00
Sodium (mg)	7.00
Potassium (mg)	340.00
Phosphorus (mg)	46.00
Copper (mg)	0.30
Selenium (µg)	0.50

(ICAR-NBAIM), Mau, Uttar Pradesh 275103, India. The commercially available *Azotobacter* (Azo) strain was obtained from Krishi Vigyan Kendra (KVK), Rajsamand, Rajasthan 313324, India. It was a liquid formulation of the Azo strain, developed by Maharana Pratap University of Agriculture and Technology (MPUAT). This formulation was checked at the Department of Biotechnology, KNHPI, Jaipur before being used in the experiments.

Isolation of new bacterial strains

The *Bacillus* species (B.st.s) was isolated from the KNHPI garden soil collected on July 21, 2023. The isolation was conducted with dilutions (up to 10^{-3}) followed by pour plate method. The Gram's staining followed by microscopic analysis showed the isolate was a Gram-positive, rod-shaped bacterium (14).

Collection of waste effluents of handmade papermaking units

The effluents of cow-dung based handmade papermaking process, namely Raw Liquor (RL) was collected for the experiments. As described in earlier literature, RL was the waste liquor isolated from the fibre-rich dewatered cow-dung residue at KNHPI (5). Freshly collected RL was used in the present study.

Procurement of seeds

Capsicum frutescens (var. NU 1919) seeds were obtained from Rajasthan Agricultural Research Institute (RARI), Durgapura, Jaipur, Rajasthan 302018, India.

Seeds sterilization method

Chilli seeds were surface sterilized with 1.5 % mercuric chloride solution for 3 min followed by 10X sterile water washing. The sterilized seeds were sown in pots using the modified method reported in literature (15).

Plant test

Experiments were conducted in plastic pots (12 cm high, 14 cm diameter and 38 cm girth) filled with air dried and sieved soil (75-120 µm mesh). Surface sterilized seeds were sown and maintained under ambient conditions. Seedlings were transplanted to field plots approximately two weeks after germination (Fig. 2).

Preparation of modified RL solutions for formulation as a biofertilizer

The BM, *Azo* and ECM strains were maintained in nutrient agar media (HiMedia, M001), Jenson's agar (HiMedia, M710) and King's B media respectively. The King's B media was prepared by dissolving glycerol (10 g/L), dipotassium hydrogen phosphate (1.5 g/L), magnesium sulphate (1.5 g/L), peptone (20 g/L) and agar (20 g/L) in distilled water at 28 °C for 48 h. The 48-h old culture broth was used to inoculate RL solution @2 % and incubated for a period of 48 h at 28 °C and 100 RPM as per the combination of cultures described below. Before using the chosen microbial consortia, the compatibility of all the selected cultures to grow together was also tested using the standard test method of inoculating the microbes into nutrient agar (NA) plates through cross streak method. Keeping in view of the extensive reports of the role of *Azotobacter* strains in improving the chilli plant's growth (16-18), the present study included a combination of *Azo* with the different microbial strains as detailed below. Since, many of our earlier studies conducted with different plants have established the biofertilizer potential of RL as compared to the normal tap water control, present study included an effort to modify RL with an aim to further improve its biofertilizer potential while considering it as a control.

- Set A: Unsterilized RL without inoculation
- Set B: RL + BM + Azo
- Set C: *RL* + *Bacillus* isolate (B.st.s) + Azo
- Set D: RL + Azo + ECM

Randomized Block Design (RBD) was used for growing plants in the field having a plot size each of 35.5 × 32 cm. The plants were grown in three different blocks and labelled with flags as three replicates of each of the three samples (Sets B, C and D) and the control (Set A) as shown in Fig. 3.

A-1	B-1	C-1	D-1
C-2	D-2	A-2	B-2
B-3	A-3	D-3	C-3

Fig. 3. Random Block Design (RBD) of the chilli plant cultivation at KNHPI campus.

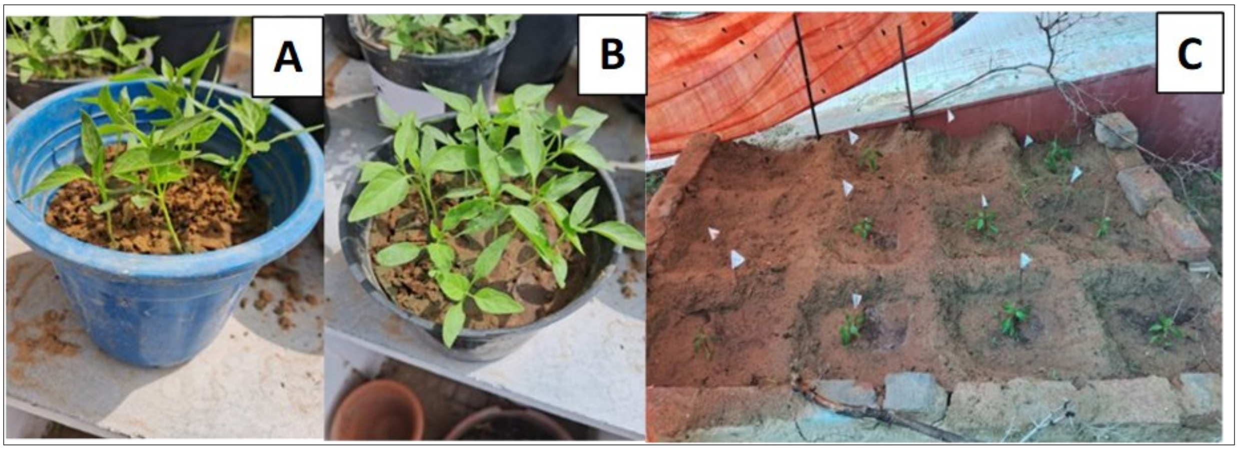

Fig. 2. (A) and (B): The germinated seedling before transferring to the field. (C) The seedlings after transfer.

Inoculation of the liquid biofertilizer into the rhizosphere of the chilli plants

A hole was created near the root zone of each plant using an iron rod and 3 mL of the prepared biofertilizer solution was applied weekly to the rhizosphere (Fig. 4).

Review of progress during the crop development period

The chilli crop growth features such as the number of leaf pairs/branches, buds, flowers, fruits and other characteristics were noted in regular intervals of one to two weeks. As and when required, an incense stick was used as an insecticide. The insecticide was formulated with neem paste and cow dung and used to ward away pests, flies, ants and mosquitoes. This was especially needed during the flowering stage of the chilli plant. Similarly, at one instance on observing a few diseases like symptoms on the leaves, a neem paste (*Azadirachtus indica*) based environment friendly organic pesticide (procured from Pinjrapole Gaushala, Sanganer, Jaipur) was sprinkled over the chilli plant leaves and the soil (Fig. 5).

Harvesting of fruits and analysis of qualitative and quantitative attributes of chillies

Chilli was harvested at various growth stages. Initially, green chillies were shade-dried to obtain red variants; later-stage fruits were allowed to mature on the plant. Each fruit was assessed for fruit length, girth, calyx and pedicel length, fresh and dry weight. Quantitative and qualitative analysis included measurements of Vitamin A, Vitamin C and Capsaicinoid content, conducted at M/S Oasis Test House Limited, Jaipur using standard protocols (HIS, IS 5838:1970 and AOAC 995.03).

Uprooting of plants and recording of observations regarding root/shoot length

Finally, the chilli plants were uprooted on 3rd June 2024 and analyzed for the root length, shoot length, air dried weight, number of branches, etc.

Fig. 5. Neem based pesticide used for sprinkling over leaves.

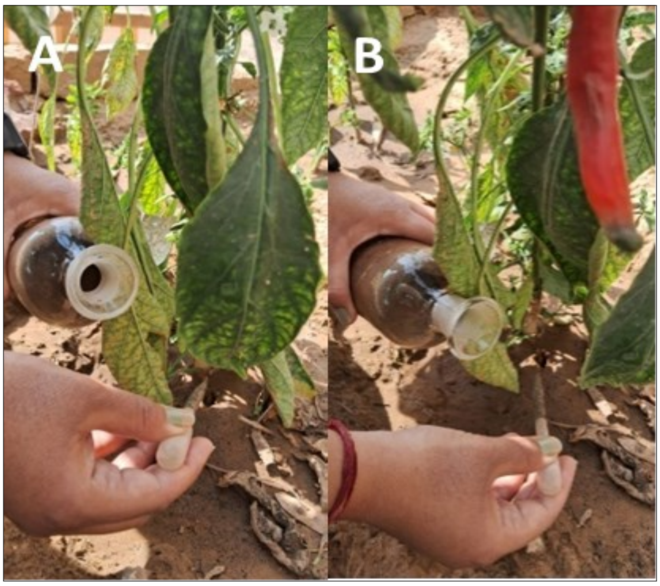

Fig. 4. Inoculation of biofertilizer samples into the rhizosphere of specific plants.

Results and Discussion

The microscopic view of the Gram-stained slides of the different PGPR strains presented in Fig. 6 shows that the *Bacillus* sp. (B.st.s) isolated from garden soil was Gram-positive, rod-shaped bacteria, arranged in chains of 3-4 bacterial cells. The BM was found to be a Gram-positive, rod-shaped bacterium having a few chains of two bacterial cells. The Azo strain was found to be Gram negative; oval shaped bacteria arranged in diplococcus pattern. The ECM was found to be Gram negative, rod shaped, spore forming bacteria arranged in chains of two cells. The colony morphology was also found to be different for all the three bacterial strains used. The *Bacillus* sp. (B.st.s) grew in the branched (rhizoid) pattern on nutrient agar plates. The BM grew in the form of thick colonies having branched edges. The Azo grew in the form of glossy white, thick colonies.

Biocompatibility of the PGPR strains used

The biocompatibility of Azo with the other selected PGPR strains was assessed using the standard cross-streak method on nutrient agar plates (Fig. 7). The results confirmed that Azo was compatible with *Bacillus megaterium* (BM), isolated *Bacillus* species (B.st.s) and *Enterobacter kobei* (ECM). This compatibility supported the formulation of multi-strain biofertilizer combinations as described in the methodology.

Review of the progress of plant growth

Plant growth was monitored on a regular basis for the number of branches, buds, flowers and fruits besides the height of all the plants individually. Accordingly, the plant height (on 50, 100, 150 and 205 DAS), number of branches per plant (on 150 and 250 DAS) and days taken to achieve first flowering stage of each set (as a mean value of all the plants of a given set) is shown in Table 2. Red chillies were also harvested from each set. Status of the chilli plant progress is shown in Fig. 8 and 9 on 40th and 147th DAS respectively.

While reviewing the plant progress, the indigenously produced insecticidal cow dung-based incense sticks were burnt under the plants to ward off insects and to use its antibacterial potential for protecting the chilli plants under study especially during the flowering stages. Similarly, the

Table 2. Effect of biofertilizer samples on growth attributes of chilli plants

Set	Treatment details	Plant height (cm)				Number of branches per plant		Days to first flowering
		Days after sowing (DAS)						
		50	100	150	205	150	250	
A	RL	23.43	36.56	45.76	55.50	21.33	32.00	41
B	RL + AR + BM	24.70	33.36	42.16	49.00	28.00	36.00	54
C	RL + AR + B.st.s	13.43	17.53	31.50	36.00	6.66	34.50	99
D	RL + AR + ECM	27.36	38.90	50.73	61.00	20.66	52.00	41

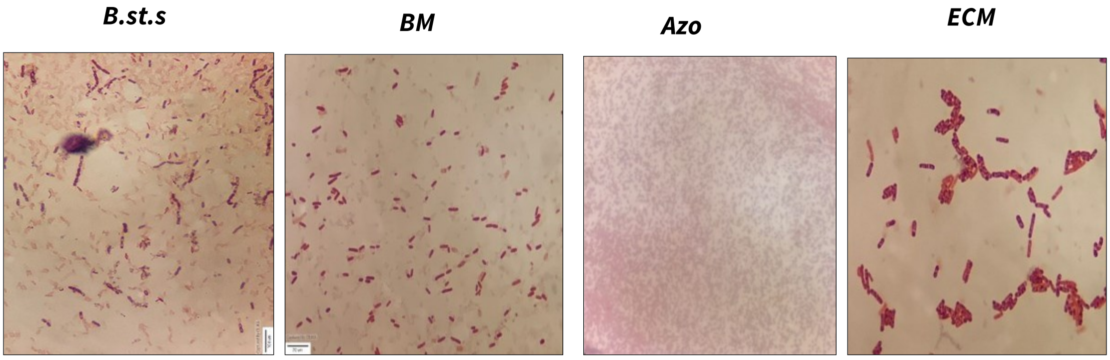

Fig. 6. Gram staining of the PGPR strains.

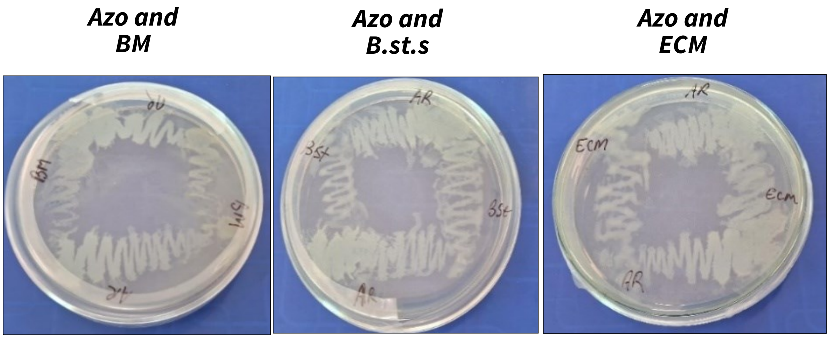

Fig. 7. Biocompatibility of the PGPR strains.

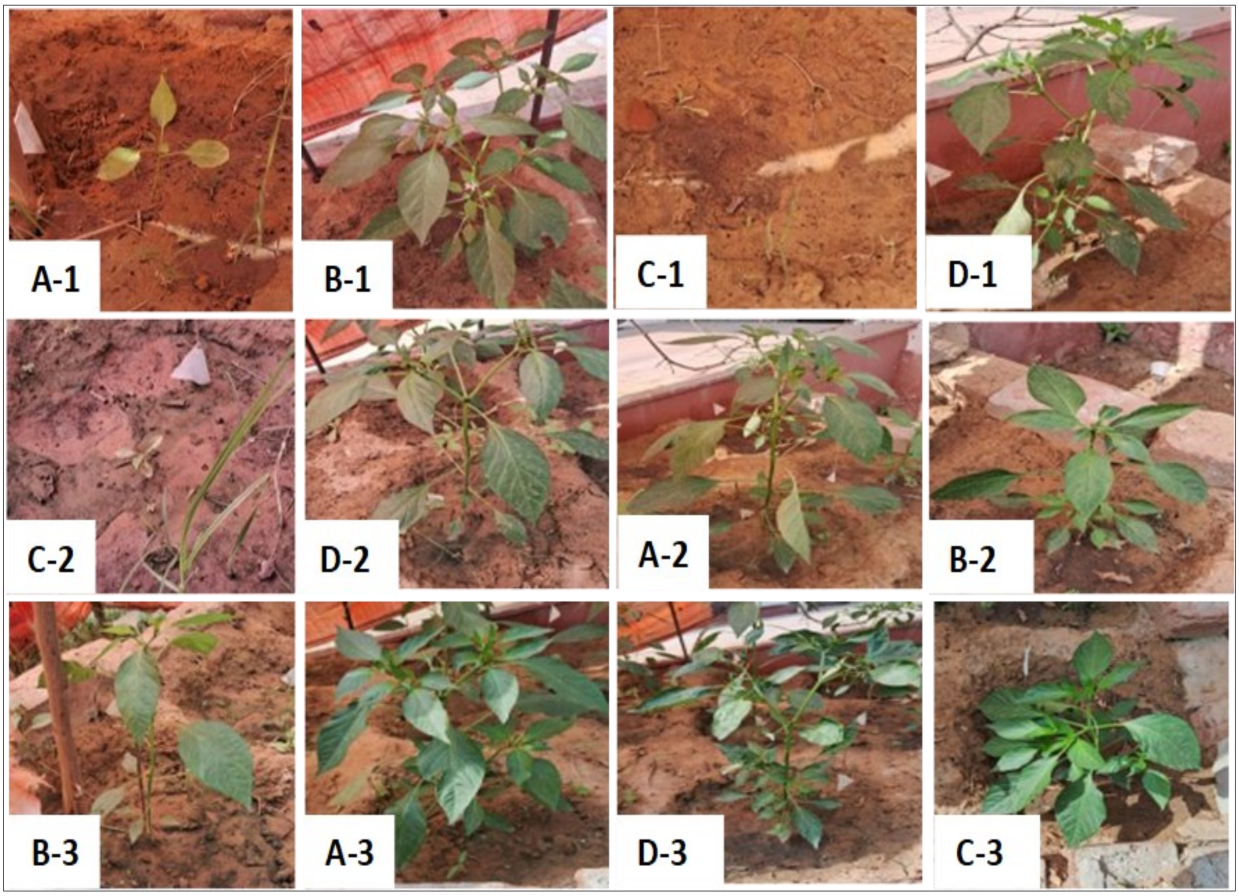

Fig. 8. Status of chilli plants in plots on 40 DAS.

Fig. 9. Status of chilli plants in the plots on 147 DAS.

neem based organic pesticide was sprinkled over the leaves to repel the nematodes, grubs, termites and mites. It had to be sprinkled three times during the budding and flowering stage of the plants. Thus, deliberate efforts were made to protect the chilli plants from diseases or any other infection without affecting the nutritional status of the plants and without introducing any chemical input to the plants.

Effect of biofertilizers used on the plant growth parameters

The effect of the liquid biofertilizer formulations on chilli plant growth was assessed by monitoring key parameters such as plant height, number of branches per plant, days to first flowering, number of buds and flowers. Data for plant height, branching and flowering for Sets A, B, C and D are presented in Table 2. Plants treated with only the RL (Set A) showed a consistent increase in plant height from 23.43 cm on 50 DAS to 55.50 cm on 205 DAS (Table 2). This suggests that the raw RL, though untreated and unsterilized did not hinder plant height growth activity. In fact, our earlier results with mung bean (*Vigna radiata*) have proved RL to be an effective biofertilizer when compared to control samples irrigated only with tap water (5). Therefore, as a furtherance of earlier findings, the present study was planned to explore the PGP effect of modified RL (by adding different PGPR strains into RL) on the chilli plants.

The chilli plants treated with Set B with a combination of RL + Azo + BM initially showed plant height growth rates comparable to the Set A, with the plant heights recorded at 24.70 and 33.36 cm on 50 and 100 DAS respectively. However, the plant height growth rate was found to be lower than Set A beyond this duration of study with the height recorded at 42.16 and 49.00 cm on 150 and 205 DAS respectively (Table 2). However, the number of branches per plant was 28.00 and 36.00 at 150 and 250 DAS respectively for Set B and this appeared to be comparable to the 21.33 and 32.00 for 150 and 250 DAS respectively for Set A. Notably, Set B plants reached first flowering later (54 DAS) compared to Set A (41 DAS). The plant growth promoting effect of four different *Bacillus* strains isolated from chilli rhizosphere as both independently and in combination on growth and physiological activities of the chilli seedling variety LCA 344 (19).

The plant height growth rate and the duration for first flowering data put-together suggest that the addition of Azo and BM (Set B) appears to have lowered the biofertilization efficiency of RL (Set A). The Azo and BM have already been proven to be mutually biocompatible strains. Also, the commercially procured Azo is a free-living nitrogen fixer. *Azotobacter* strains have been reported to be very effective for promoting the growth of various plants including Chilli plants. Hence, these are unlikely to the causal factor for the lowered productivity. It may be inferred that either BM by itself or due to its unfavourable combination with Azo or with the inherent bacteria of unsterilized RL is the causal factor, which instead of being synergistic, might have acted as an antagonist hindering the chilli plant growth productivity.

In Set C, the chilli plants were treated with a combination of RL, Azo and B.st.s. The results documented in Table 2 show that the plant heights for Set C were 13.43, 17.53, 31.50 and 36.00 cm at 50, 100, 150 and 205 DAS respectively. This data can be used to infer that there is time-delay for the growth in height of the plant when compared to that of Sets A and B. The number of branches per plant was also found to be a meagre 6.66 till 150 DAS followed by a spurt to 34.50 at 250 DAS. The duration of first flowering at 99 DAS for Set C was significantly higher than the duration observed for Set A (41 DAS) and Set D (54 DAS). These observations also reinforce the earlier observation of a time-delay in chilli plant productivity when RL is used with Azo and B.st.s as the biofertilizer formulation. As Azo and B.st.s have already been found to be biocompatible bacterial species, the time-delay observed for onset of growth of the chilli plants is likely to be due to the action of either B.st.s by itself or due to its unfavourable combination with Azo which is the causal factor for delaying the growth, leading to reduced height growth rate, reduced number of branches and increased days to flowering in the chilli plants studied.

In contrast to the above observations, the experiments with Set D utilizing a formulation of RL + Azo + ECM showed superior plant growth productivity than Sets A, B and C. For example, with Set C, the plant heights recorded were 27.36, 38.90, 50.73 and 61.00 cm at 50, 100, 150 and 205 DAS, whereas the number of branches was 52.0 at 250 DAS and the days to first flowering was 41 DAS, all significantly superior to Sets A, B and C. These results show that ECM enhances the biofertilization potential of Azo in RL. This can also be used to conclude that the relatively poor biofertilization performance observed in Sets B and C is not due to the combination of BM and B.st.s respectively with AR but most probably it may be due to these PGPR strains individually. Overall, these experiments show that the liquid biofertilizers prepared by modifying RL with addition of Azo and ECM to be the best plant growth enhancer for chilli plants.

These observations of favourable growth enhancement activity by Azo and ECM are supported by a few literature reports. *Azotobacter* to be most prevalent species in the rhizosphere soil of chilli plants of Cuddalore district, Tamil Nadu, India and the species induces Indole Acetic Acid (IAA) production and nitrogen fixation, thereby aiding the growth of chilli plants (17). The beneficial effects of nitrogen fixing *Azotobacter* and *Azospirillum* on growth and yield of chilli plants. They have however reported that the results with *Azospirillum* were better than the *Azotobacter* sp. (18). The genus *Enterobacter* is known to enhance soil nitrogen content, microbial diversity and PGP traits such as IAA production, phosphate solubilization and antagonism against pathogens (20, 21). Collectively, these findings validate the superior performance of Set D. Among all combinations tested, the RL-based biofertilizer incorporating biocompatible Azo and ECM is the most effective in promoting chilli plant growth.

Effect of biofertilizers on quality and quantity of chillies harvested from different sets

During the plant growth, red chillies were harvested from different plants. The red chillies harvested were assessed for their number as well as the characteristic features involving length, circumference, length of calyx + pedicel, fresh weight and OD weight as given in Table 3. Some of the red chillies harvested on different days are shown in Fig. 10. Later, the red chillies of different sets were also evaluated for the basic biochemical attributes as tabulated in Table 4. Finally, the plants were uprooted on 3rd June 2024 (Fig. 11) and observations were recorded as shoot length, root length and weight of the plant. The average values along with the standard deviation are given in Table 4. Fruit yield (in g) per plant was also calculated as shown in Table 4. While going through the qualitative and quantitative aspects of harvested chillies of different Sets from the chilli plants grown in Randomly Block Designs, it can easily be inferred that the Set D had resulted not only into the highest number of chilies, but it also produced chillies with maximum fruit length, girth, fresh weight and OD weight. However, the pedicel + calyx length was found to be the longest in the case of Set-A where only the un-autoclaved RL was used as such (*i.e.* without any additional bacterial inoculums) for enriching the rhizosphere of the selected chilli plants.

As per the biochemical characterization of the red chillies harvested from different sets of plants, it can be seen from the Table 5 that the Set A (167.79 mg/100g) and Set D (167.39 mg/100g) had shown almost equal values of Vitamin C content. Vitamin C content reflects the antioxidant properties of the fruit which provide defence against the substances that can cause cancer and helps in slowing down the ageing process (22, 23). Although Valverde and Santos ‘s team have reported 121.5 mg/100g of Vitamin C in *C. frutescens*, the present study has shown much higher contents of Vitamin C in all the sets. Thus, the RL solution both in natural and modified form has resulted in the enhancement of Vitamin C contents of the red chillies (11, 24).

Although Vitamin C content was almost equal in Set A and Set D but the Vitamin A content was remarkably high in Set A (482 IU/g) as compared to the other sets. The Set B (374.38 IU/g) and Set C (370.82 IU/g) were found to have the Vitamin A content in similar range. Whereas the Set D (388.29 IU/g) had an intermediate amount of Vitamin A content. While referring to the biochemical characteristics of chilli pepper, it is important to note here that the pungency of peppers is linked to the presence of capsaicin. The capsaicin concentration (and consequently pungency) is expressed by a sensory scale called Scoville Heat Units (SHU), where values range from zero for milder peppers to 1 million SHU for the spicier varieties. Capsaicin is a compound responsible for the sensation of heat and burning (11, 23). Therefore, while observing the capsaicinoids content, it was found to be maximum (67793.18 SHU) in the case of red chillies harvested from Set C. Therefore, enriching the plants of Set C with the

Table 3. Effect of various biofertilizer samples on chilli growth parameters

Parameters	Set-A				Set-B				Set-C			Set-D				Overall best values observed in the set
	A-1	A-2	A-3	Average value of Set-A	B-1	B-2	B-3	Average value of Set-B	C-2	C-3	Average value of Set-C	D-1	D-2	D-3	Average value of Set-D	
No. of fruits	7	30	32	23	36	21	12	23	6	11	8.50	24	25	29	26	**26 (Set-D)**
Girth (cm)	3.48± 0.17	3.41± 0.74	3.49± 0.85	3.46± 0.04	3.53± 0.76	3.49± 0.85	3.07± 1.14	3.49± 0.41	3.20± 1.10	3.42± 1.10	3.31± 0.15	3.62± 0.52	3.85± 0.77	3.35± 0.97	3.61± 0.25	**3.61± 0.25 (Set-D)**
Fruit length (cm)	5.60± 0.87	8.12± 2.54	8.17± 2.07	7.29± 1.46	8.17± 2.07	7.83± 2.49	5.56± 1.36	6.59± 1.14	7.92± 1.72	6.00± 0.94	6.96± 1.35	6.92± 2.16	8.50± 1.61	7.46± 2.32	7.63± 0.80	**7.63± 0.80 (Set-D)**
Pedicle + calyx (cm)	3.28± 0.40	3.49± 1.04	3.53± 1.06	3.43± 0.13	3.53± 1.06	3.66± 1.06	2.75± 0.69	2.99± 0.58	3.45± 1.05	2.63± 0.73	3.04± 0.57	3.12± 0.85	3.85± 0.94	2.84± 0.94	3.27± 0.52	**3.43± 0.13 (Set-A)**
Fresh weight (g)	1.49± 0.38	3.22± 1.08	3.55± 1.83	2.75± 1.10	3.55± 1.83	3.42± 2.16	2.25± 2.0	2.72± 0.60	2.13± 1.07	2.98± 1.43	2.55± 0.60	3.13± 0.76	3.73± 1.48	2.96± 1.73	3.27± 0.40	**3.27± 0.40 (Set-D)**
Dried weight (g)	0.27± 0.11	0.84± 0.41	1.15± 0.38	0.75± 0.45	0.86± 0.35	0.66 ± 0.32	0.64± 0.29	0.72 ± 0.12	0.39± 0.02	0.86± 0.36	0.62± 0.33	092± 0.18	1.17± 0.32	0.69± 0.43	0.93± 0.24	**0.93± 0.24 (Set-D)**

Fig. 10. Red chillies harvested from different plants at different points of time.

Table 4. Effect of biofertilizer samples used on plant growth parameters of chilli plants of different sets

Parameters	Set-A	Set-B	Set-C	Set-D	Overall best values observed in the set
Fruit yield/plant (gm)	73.54 ± 55.31	75.54 ± 50.50	22.78 ± 14.14	84.73 ± 9.11	**84.73 g (Set-D)**
SL of uprooted plants (cm)	53.9 ± 29.83	52.6 ± 2.85	42.15 ± 3.04	56.7 ± 8.16	**56.70 cm (Set-D)**
RL of uprooted plants (cm)	22.63 ± 0.15	23.5 ± 2.96	10.25 ± 10.25	22.56 ± 3.16	**22.63 cm (Set-A)**

SL-Shoot length, RL-Root length.

Table 5. Effect of biofertilizer samples on biochemical attributes of the chilli harvest

Sets	Biofertilizer details	Capsaicinoids Content (SHU)	Capsaicinoids Content (%w/w)	Vit A (IU/gm)	Vit C mg/100gm
A	RL	21768.39	0.145	482.22	167.79
B	RL + AR + BM	31930.52	0.213	374.38	159.79
C	RL + AR + B.st.s	67793.18	0.452	370.82	155.01
D	RL + AR + ECM	44265.13	0.295	388.29	167.30
Overall best values observed in different sets		67793.18 (Set-C)	0.452 (Set-C)	482.22 (Set-A)	167.79 (Set-A)

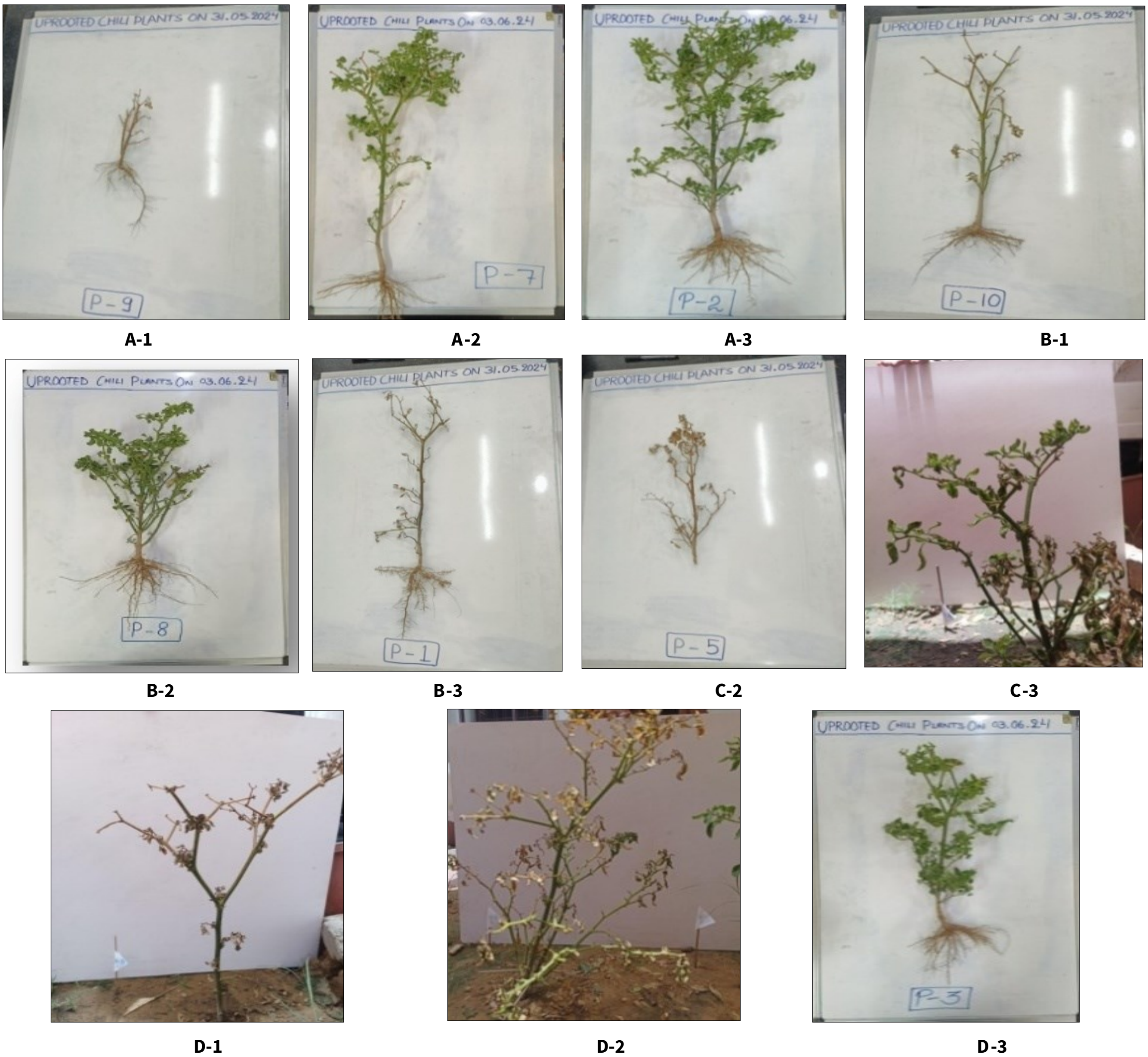

Fig. 11. Uprooted chilli plants.

biofertilizer samples prepared using a combination of *Azotobacter* sp. and *Bacillus* sp. (B.st.s) resulted in the highest content of capsaicinoids in the chilli harvest of these plants. Among the remaining three sets, Set D recorded the highest content of capsaicinoids content (42265.13 SHU) as compared to the Set A (21768.39 SHU) and Set B (31930.52 SHU). So, pungency was found to be in the decreasing order as Set C>Set D>Set B>Set A.

Overall, the RL-based biofertilizer containing *Azotobacter* and *Enterobacter* (Set D) demonstrated superior performance in terms of both plant growth and fruit yield. These findings are consistent and documented high capsaicin levels in *C. frutescens* and *C. baccatum var. pendulum* (25). The present study underscores the potential of PGPR-enriched RL biofertilizers to enhance both the quantitative and qualitative traits of chilli cultivation.

Conclusion

Based on the findings of this study, it can be concluded that the raw effluent (RL) from the handmade paper manufacturing process-particularly when cow dung is used as a raw material-possesses considerable potential as a biofertilizer. The modified RL solutions *i.e.* the liquid biofertilizer samples thus developed have resulted into higher fruit yields and capsaicinoids content when the rhizospheres of chilli plants were inoculated with them. Such use of effluents can not only help in reducing the pollution problems caused using chemical fertilizers in agriculture, but it can also help in sustainable management of the handmade paper industry waste besides creating additional income generation opportunities to the gaushalas and handmade paper manufacturers. These results advocate for the integration of such eco-friendly biofertilizers in sustainable agriculture and circular economy models.

Acknowledgements

Thanks are due to the Directorate of Science and Technology, Khadi & Village Industries Commission (KVIC), Mumbai for financial assistance in the form of an S&T, KVIC project to KNHPI, Jaipur. Thanks are also due to the JRF, Miss Priyanka

Jagetiya and Miss Divya Harwani, Project Assistant, S&T, KVIC project.

Authors' contributions

SN is the research scholar and prepared the manuscript draft. RM edited the manuscript and critically examined the ongoing studies as the Director of the Research Centre. SC designed the experiments, co-supervised the research work (External Supervisor) and edited the manuscript. MR supervised the research work and edited and approved the final draft of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interests to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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