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RESEARCH ARTICLE

Impact of bioinoculants on seed germination, early growth and rhizospheric microbial community of *Moringa oleifera* L.

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Abstract

An experimental study was conducted under the supervision of the Department of Silviculture and Agroforestry, College of Horticulture and Forestry, ANDUAT University, Uttar Pradesh, to assess the Impact of bioinoculants on seed germination, early growth and rhizospheric microbial community of *Moringa oleifera* L. The duration of the experiment lasts for one season. In this the seeds were initially germinated under laboratory conditions and later transferred to polybags in a nursery setting. The study explored the effects of both individual and combined applications of microbial bioinoculants such as *Azotobacter* (10 mL), *Pseudomonas* (10 mL) and seaweed (5 mL) extract using a Completely Randomized Design (CRD) for germination and Randomised Block design (RBD) for growth and microbial count with 3 replications. Results revealed that integrated treatments significantly outperformed individual applications and the control group across several germination and vigour-related parameters. Notable improvements were observed in emergence rate (85.93 %), germination percentage (88.66 %), mean germination time (lowest 5.66 days), speed of germination (4.5), mean germination rate (0.216), mean daily germination (0.833), peak value (1.6), germination value (30.42), seedling vigour (87.96) and overall germination efficiency along with growth and enhanced microbial community (*Azotobacter*- 9.397×10^6 and *Pseudomonas* spp.- 9.297×10^5). The best-performing combination of *Azotobacter* + *Pseudomonas* + Seaweed (T_7) displayed enhanced seed vigour and superior morphological traits such as greater plant height (52.5 cm), stem thickness (5.86 mm) and biomass accumulation (fresh 23.91 g and dry weight 6.46 g of plants) and increased microbial population (Bacteria- 13.797×10^5, Actinomycetes- 10.799×10^6, Fungi-7.847×10^4). The synergistic effect of combined bioinoculants suggests a strengthened microbial interaction that enhances nutrient availability, hormonal stimulation and root microbe signalling. These findings underscore the potential of integrated bio stimulant strategies for boosting early-stage development of *Moringa oleifera*, offering a promising, sustainable approach for improving nursery practices and promoting eco-friendly agroforestry interventions.

Keywords: *Azotobacter*; germination; *Moringa oleifera*; microbial count; *Pseudomonas*; seaweed

Introduction

Moringa oleifera L. is a widely cultivated multipurpose tree species valued for its rapid growth, drought tolerance and adaptability to diverse agroclimatic conditions. Originally native to the Indian subcontinent, it has become naturalised across many tropical and subtropical regions of the world (1). The tree is well recognised for its exceptional nutritional composition, with leaves, pods and seeds rich in proteins, vitamins and minerals, while its roots and extracts are applied in traditional medicine and industrial uses (2, 3). For these reasons, it is often referred to as a "miracle tree", reflecting its multifunctional role in food, health and livelihood security. Despite being widespread, moringa seedlings often poses significant challenges in the germination and early establishment due to factors like variable seed viability, sensitivity to moisture fluctuations and vulnerable to fungal and insect attacks in the nursery stage. The hard seed coat sometimes hampers uniform germination; it requires pre-sowing treatments like soaking or scarification to enhance emergence. Irregular watering or poor drainage can lead to seed rot or damping-off disease, while inadequate soil aeration also restricts root development. Moreover, maintaining optimal temperature and light conditions is crucial, as excessive shade or direct exposure can stress young seedlings. Therefore, effective nursery management including careful monitoring, use of well-drained fertile media, pest and disease control and regular thinning or pricking for healthy growth and uniform stand establishment is necessary.

M. oleifera is recognised as a multifunctional species of considerable importance in agroforestry systems, owing to its rapid establishment, substantial foliar biomass and deep-rooted architecture, which collectively enhance soil fertility, provide windbreak functions, support intercropping through moderated shade and contribute to the restoration of degraded lands (4). Recent investigations emphasise that sustainable propagation of *M. oleifera* under low-input environments requires strategies that reliably strengthen early growth and nutrient uptake (5, 6). Emerging

evidence from the past five years indicates that microbial bioinoculants including plant-growth-promoting (PGPR) rhizobacteria, arbuscular mycorrhizal fungi and multi-strain microbial consortia substantially enhance germination, seedling vigour, nutrient assimilation and rhizosphere microbial dynamics across forestry and agroforestry species, including *M. oleifera* (7–9).

Bioinoculants represent one such strategy, offering a biologically based and environmentally friendly alternative to chemical fertilisers. Microbial inoculants such as *Azotobacter* and *Pseudomonas* enhance nutrient availability through nitrogen fixation, phosphorus solubilisation and stimulation of beneficial microbial populations, while seaweed extracts provide growth-promoting hormones and micronutrients (10, 11). Thus, the integrated use of these inputs has been shown to improve seed germination, biomass accumulation, metabolism uptake and soil microbial activity, thereby strengthening the plant soil interface.

Considering these aspects, the present investigation examines the effects of selected bioinoculants on seed germination, early seedling development and the enhancement of soil microbial populations in *M. oleifera*, with the broader aim of formulating sustainable, low-input propagation strategies. Growing concerns over the environmental and economic implications of synthetic fertilizers have accelerated the transition toward biologically derived organic alternatives, including microbial inoculants and seaweed extracts. These inputs improve nutrient use efficiency, enhance the microbial diversity and strengthen soil health, thereby supporting agricultural productivity while maintaining ecological integrity. The integration of *M. oleifera* cultivation with bioinoculant-based management practices therefore presents a feasible approach for developing resilient, resource-efficient and environmentally aligned agroforestry systems (12–16).

Materials and Methods

Experimental materials

Sustainable higher quality mature, viable superior local variety seeds of *M. oleifera* were collected from local market and treated to identify initially the germination and growth of seedlings. Bioinoculants including *Azotobacter chroococcum* (ABA-1), *Pseudomonas fluorescens* (BAU_D2) as liquid form were procured from Microbiology lab of ANDUAT and seaweed extract was brought from IFFCO certified suppliers to ensure microbial integrity and formulation quality. The experiment was designed with 8 distinct treatments in which the *Moringa* seeds were treated with different bioinoculants to see its impact on germination, growth efficacy and its application in soil. The following are the different treatments (Table 1).

Seed pre-treatment and inoculation

In advance of sowing, seeds underwent hydropriming by soaking in distilled water for 14–16 hr to facilitate moisture imbibition and then seeds were surface sterilised with recommended wettable fungicides solution for about 20–30 min and shade dry them in a thin layer until they are surface dry to prevent microbial contamination. Subsequently, seeds were treated with a bioinoculant *Azotobacter* (10 mL), *Pseudomonas* (10 mL) and seaweed (5 mL) extract suspension which was diluted by mixing water 10 times of the volume of inoculants (10 mL inoculant per 20 g seed) and soaked for 2–4 hr (Fig. 1), air-dried under shade for 1 hr to maintain microbial viability and used for sowing.

The seeds were subjected to germination in pro tray filled with a standardized oven sterilized (180 °F/82 °C for 30–40 min) medium composed of soil, sand and well-decomposed farmyard manure (2:1:1) at room temperature in lab. The blended soil mixture tends to be sandy loam, well drained with pH of 7.6, soil EC of 0.29 dS/m and soil OC is 0.46 %. A total of 60 seeds per treatment were sown across three replicates (20 seeds per tray), at a uniform depth of 2–2.5 cm (Fig. 2). Control groups (untreated seeds) were also established. Moisture was maintained uniformly across all treatments throughout the observational period (Fig. 3).

Table 1. Different treatments used in the experiment

Treatment Symbol	Treatments
T_0	Control (Soil + Sand + FYM)
T_1	Soil + Sand + FYM + *Azotobacter*
T_2	Soil + Sand + FYM + *Pseudomonas*
T_3	Soil + Sand + FYM + Seaweed extract
T_4	Soil + Sand + FYM +*Azotobacter* + *Pseudomonas*
T_5	Soil + Sand + FYM + *Azotobacter* + Seaweed extract
T_6	Soil + Sand + FYM + *Pseudomonas* + Seaweed extract
T_7	Soil + Sand + FYM + *Azotobacter* + *Pseudomonas* + Seaweed extract

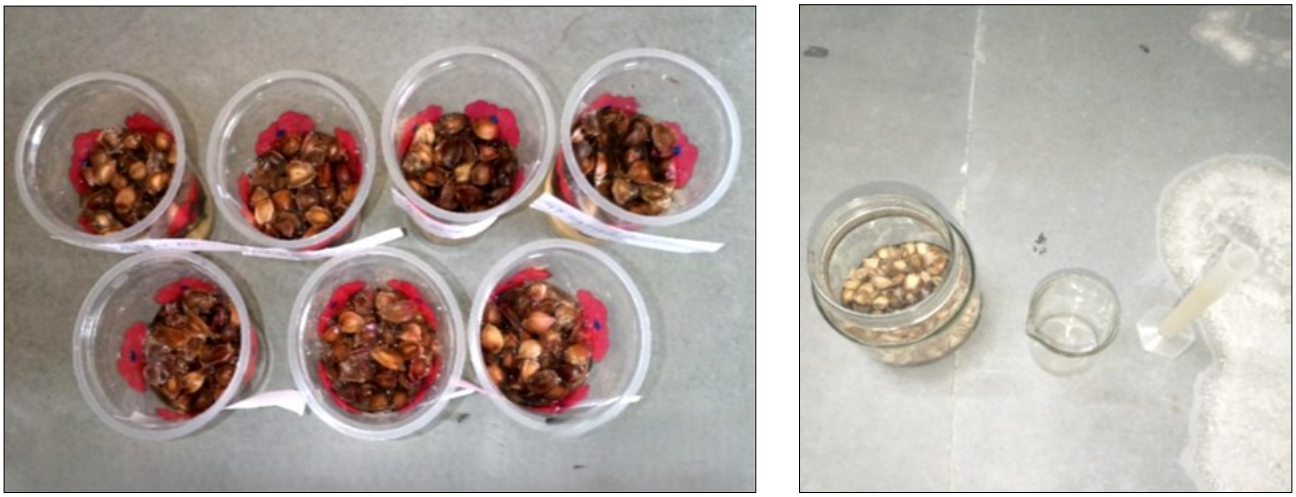

Fig. 1. The seeds were soaked after hydro primed with the respective solution.

Fig. 2. Sowing of seeds.

Fig. 3. Maintaining moisture.

Observation parameters and analytical methods

Seed germination and emergence metrics

Germination percentage (G %):

$$G\,\% = \frac{\text{Number of normal seedlings}}{\text{Total seeds sown}} \times 100$$

Rate of emergence (RE):

$$RE = \frac{\text{Seedlings emerged in 5 days}}{\text{Seedlings emerged in 15 days}} \times 100$$

Germination value (GV):

$$GV = GP \times \frac{\text{Cumulative GP}}{\text{Days since sowing}}$$

Mean daily germination (MDG):

$$MDG = \frac{\text{Total germinated seeds}}{\text{Total number of days}}$$

Mean germination time (MGT):

$$MGT = \sum \frac{(n \times d)}{n}$$

Where *n* = number of seeds germinated on day *d*.

Speed of germination (SG):

$$SG = \sum \frac{n}{d}$$

Where, *n* = number of seeds germinated on day *t* and *d* = day of observation.

Peak value (PV):

$$PV = \frac{\text{Highest germination count}}{\text{Days to peak}}$$

Mean germination rate (MGR):

$$MGR = \frac{1}{\text{Mean Germination Time}}$$

Seedling vigour index (17)

SVI-I = Germination (%) × Mean seedling length (cm)

SVI-II = Germination (%) × Mean seedling dry weight (g)

2.4 Growth Attributes

Plant height (cm)

Plant height, a key indicator of vegetative growth, was measured from the soil surface to the apex of the main stem using a measuring scale. Data were recorded in centimetres from five randomly selected plants per treatment and the average was calculated to represent each treatment.

Plant stem diameter (mm)

Plant diameter, indicating stem thickness and structural strength, was measured 5 cm above the soil surface using a screw gauge. Measurements were taken from five randomly selected plants per treatment and the mean diameter was recorded in millimetres (mm).

Nutrient uptake NPK and % concentration

Nutrient uptake of nitrogen (N), phosphorus (P) and potassium (K) was assessed to evaluate the plant's nutrient absorption efficiency. After oven-drying, plant samples were digested using standard procedures and NPK concentrations were determined. Nutrient uptake was calculated and expressed in milligrams per plant, while nutrient concentration was recorded as a percentage (%) of dry weight.

Fresh weight (g/plant)

Fresh weight, representing the aboveground biomass and water content, was measured at the time of harvest. Selected plants were gently uprooted, washed to remove soil and weighed immediately using a digital analytical balance. The average fresh weight per treatment was recorded in grams (g).

Dry weight (g/plant)

Dry weight, indicating the plant's total biomass excluding water content, reflects organic matter accumulation. After fresh weight assessment, samples were oven-dried for 72 hr until a constant weight was attained, then weighed using a precision digital balance and the results were recorded in grams (g).

Microbial population count

Microbial population in soil is estimated using the serial dilution and plate count method, a standard 10 folds (1:10) initially 10^{-1} dilution and the process repeated several times till 10^{-6}times, where a soil sample is diluted, plated on nutrient agar (NA-media for bacteria *Azotobacter* and PSB) (Fig. 4) and PDA for fungi and incubated at 28–30 °C for 5 days in case of *Azotobacter* whereas for PSB it is incubated at 28–30 °C for 7 days for better colony formation. The

Fig. 4. Assess of microbial population count.

resulting colonies represent cultivable microbes, allowing calculation of colony-forming units (CFUs) per gram of soil. This method reflects microbial abundance but excludes non-culturable organisms, offering only a partial view of soil microbial diversity.

Results

Effect on germination attributes of *M. oleifera* plant

Germination percentage

Germination percentage reached its maximum achieving 88.66 % observed under the triple combination treatment involving *Azotobacter*, *Pseudomonas* and seaweed extract, marking a substantial improvement over the control (60.12 %). Individual applications yielded moderate enhancements: seaweed extract (75.46 %), *Pseudomonas* (72.31 %) and *Azotobacter* (68.73 %). Dual combinations also demonstrated synergistic effects offering significant improvements, with *Azotobacter* + *Pseudomonas* (83.91 %) and *Pseudomonas* + seaweed extract (81.80 %), affirming the potency and efficacy of microbial partnerships in promoting successful germination (18, 19) (Table 2 and Fig. 5).

Seed emergence

Seed emergence followed a similar trend, with the triple treatment blending *Azotobacter*, *Pseudomonas* and seaweed extract achieving the highest emergence rate (85.93 %), far surpassing the control group's rate of 42.33 %. Among individual treatments, seaweed extract containing betaine and mannitol showed the most promising value with 64.16 %, followed by *Pseudomonas* (58.43 %) and *Azotobacter* (50.26 %), corroborating recent findings on the stimulatory role of seaweed-derived bio stimulants in early sprouting and stress resilience (20) (Table 2 and Fig. 5).

Mean germination time (MGT)

Mean Germination Time (MGT) marked reduction with microbial intervention (Table 2 and Fig. 5). The trio-treated seeds germinated in just 5.66 days, significantly quicker than the control 8.50 days likely due to enhanced microbial action improving nutrient dynamics (21). Whereas in dual integration it was recorded lowest (6.00 days) in (T_4) *Azotobacter* + *Pseudomonas* and among individual treatment only seaweed extract records the lowest 6.93 days.

Germination speed and average germination rate

Germination speed and average germination rate were fastest under the triple application (4.5 and 0.216), indicative of robust, efficient sprouting (Table 2 and Fig. 5). Dual inoculations performed moderately well, while control seeds, at 3.03 and 0.118 respectively, revealed limited intrinsic germinative potential.

Table 2. Illustrating the germination-based attributes of *M. oleifera* plant

Treatment	Code	Rate of emergence	Germination (%)	Mean germination time (days)	Speed of germination	Mean germination rate	Mean daily germination	Peak value	Germination value	Seedling vigour
Control	T_0	42.33 ± 1.241	60.12 ± 1.732	8.50 ± 0.231	3.03 ± 0.088	0.118 ± 0.003	0.60 ± 0.000	0.5 ± 0	14.53 ± 0.433	59.83 ± 1.732
Azotobacter	T_1	50.26 ± 1.184	68.73 ± 1.588	7.20 ± 0.173	3.36 ± 0.088	0.136 ± 0.003	0.667 ± 0.033	0.7 ± 0	16.56 ± 0.376	68.46 ± 1.588
Pseudomonas fluorescens	T_2	58.43 ± 2.021	72.31 ± 2.483	7.10 ± 0.231	3.51 ± 0.115	0.142 ± 0.004	0.667 ± 0.033	0.867 ± 0.033	18.12 ± 0.635	71.89 ± 2.483
Seaweed extract	T_3	64.16 ± 2.973	75.46 ± 3.493	6.93 ± 0.318	3.74 ± 0.173	0.152 ± 0.007	0.733 ± 0.033	0.9 ± 0.058	19.66 ± 0.895	74.96 ± 3.435
Azotobacter* + *Pseudomonas	T_4	74.10 ± 1.270	83.91 ± 1.443	6.00 ± 0.115	4.16 ± 0.088	0.185 ± 0.003	0.767 ± 0.033	1.367 ± 0.033	26.21 ± 0.462	83.5 ± 1.443
***Azotobacter* + Seaweed extract**	T_5	70.50 ± 1.617	79.36 ± 1.819	6.26 ± 0.145	3.83 ± 0.088	0.169 ± 0.003	0.733 ± 0.033	1.133 ± 0.033	22.93 ± 0.549	78.83 ± 1.819
***Pseudomonas* + Seaweed extract**	T_6	72.63 ± 2.107	81.80 ± 2.367	6.10 ± 0.173	4.03 ± 0.115	0.176 ± 0.005	0.767 ± 0.033	1.233 ± 0.033	24.59 ± 0.693	81.36 ± 2.338
***Azotobacter* + *Pseudomonas* + Seaweed extract**	T_7	85.93 ± 2.974	88.66 ± 3.089	5.66 ± 0.203	4.5 ± 0.173	0.216 ± 0.007	0.833 ± 0.033	1.6 ± 0.058	30.42 ± 1.039	87.96 ± 3.031
SEm (±)		2.04	2.356	0.207	0.121	0.003	0.031	0.037	0.672	2.333
CD (P = 0.05)		6.17	7.125	0.626	0.367	0.007	0.094	0.113	2.031	7.054

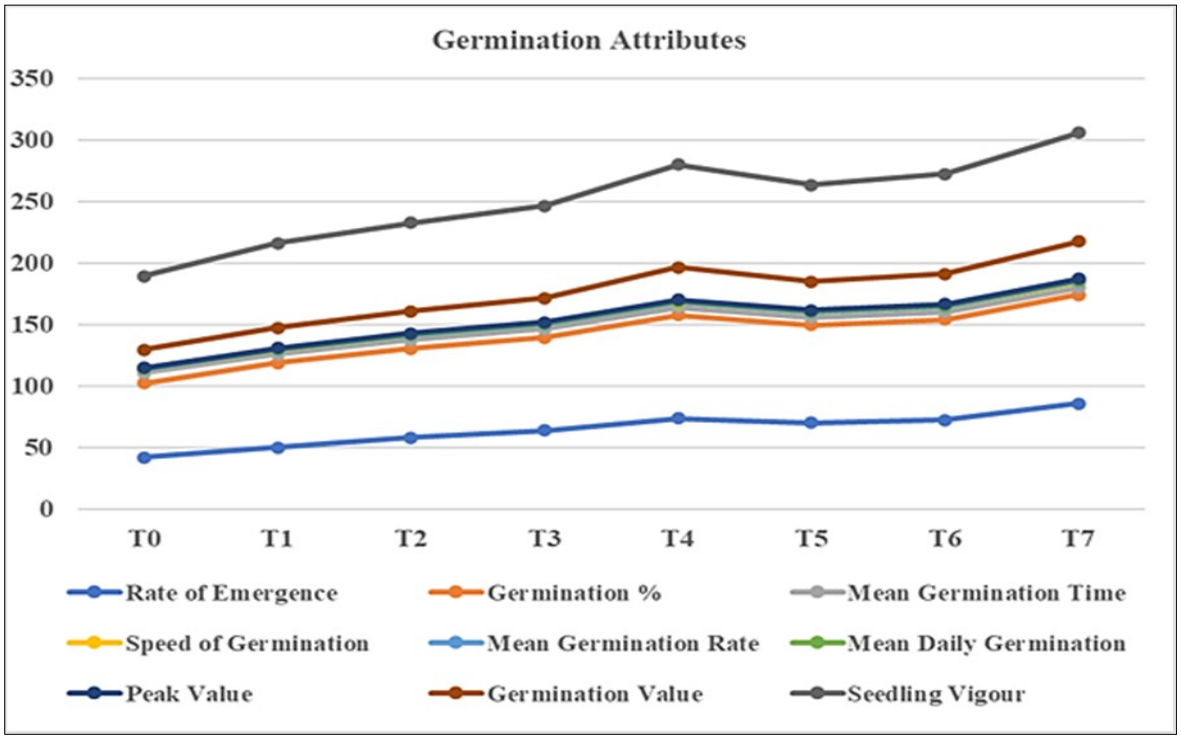

Fig. 5. Graph illustrating the germination attributes of *M. oleifera* plant.

Mean daily germination and peak value

Mean daily germination (0.83) and peak value (1.6) were also highest in the triple combination treatment, suggesting not only faster but more uniform germination critical for consistent field growth.

Germination value (GV)

The germination value (GV), combining speed and vigour metrics, peaked at 30.42 in the triple mix integration in T_7treatment, more than double the control's GV of 14.53 (Table 2 and Fig. 5). Treatments with seaweed in dual setups showed notable improvements, further validating its role in boosting seedling vitality through enhanced hydration and hormone signalling (18, 22).

Seedling vigour

Lastly, seedling vigour an indicator of long-term plant health was at its highest (87.96) in the triple formulation of *Azotobacter* + *Pseudomonas* + Seaweed extract (T_7) containing betaine and mannitol Sea derived bioactive compounds stimulating plant metabolism, increases the root-shoot vigour that results in lush foliage growth leading to more flowering and fruiting and lowest in control (T_0) than the dual and single integration treatment (Table 2 and Fig. 5).

Effect on growth attributes and nutrient uptake of *M. oleifera* plant

Among the treatments, the combined application of *Azotobacter* + *Pseudomonas* + Seaweed extract (T_7) yielded the highest values across all measured parameters, with a plant height of 52.5 ± 1.819 cm, stem diameter of 5.86 ± 0.203 mm, fresh weight of 23.91 ± 0.829 g/plant and dry weight of 6.46 ± 0.203 g/plant (Table 3 and Fig. 6). This treatment also recorded the highest nitrogen, phosphorus and potassium uptakes 113.69 ± 3.926 mg/plant, 14.21 ± 0.491 mg/plant and 89.14 ± 3.089 mg/plant respectively corresponding to elevated nutrient concentrations of 1.76 %, 0.22 % and 1.38 % (Table 4 and Fig. 7).

In contrast, the control treatment exhibited the lowest growth and nutrient uptake values, including a plant height of 32.49 ± 0.938 cm, stem diameter of 3.86 ± 0.088 mm, fresh weight of 11.07 ± 0.32 g/plant, dry biomass of 3.96 ± 0.088 g/plant and minimal NPK uptakes (53.46 ± 1.53 mg/plant, 7.12 ± 0.203 mg/plant and 42.76 ± 1.241 mg/plant respectively).

Table 3. The growth parameter of *M. oleifera* plant

Treatment	Treatment code	Plant height 90 DAS	Stem diameter 90 DAS	Fresh weight (g/plant)	Dry weight (g/plant)
Control	T_0	32.49 ± 0.938	3.86 ± 0.088	11.07 ± 0.32	3.96 ± 0.088
Azotobacter	T_1	41 ± 0.947	4.76 ± 0.088	15.26 ± 0.355	5.04 ± 0.231
Pseudomonas	T_2	38.19 ± 1.325	4.36 ± 0.145	13.30 ± 0.459	4.66 ± 0.088
Seaweed extract	T_3	40 ± 1.848	4.56 ± 0.203	14.43 ± 0.667	4.91 ± 0.173
Azotobacter* + *Pseudomonas	T_4	45.59 ± 0.788	5.16 ± 0.088	17.52 ± 0.303	5.61 ± 0.115
***Azotobacter* + Seaweed**	T_5	47.19 ± 1.088	5.36 ± 0.145	20.02 ± 0.465	5.81 ± 0.173
***Pseudomonas* + Seaweed**	T_6	46 ± 1.328	5.26 ± 0.145	18.85 ± 0.546	5.66 ± 0.145
***Azotobacter* + *Pseudomonas* + Seaweed**	T_7	52.5 ± 1.819	5.86 ± 0.203	23.91 ± 0.829	6.46 ± 0.203
SEm (±)		1.373	0.15	0.538	0.167
CD (*P* = 0.05)		4.206	0.461	1.647	0.511

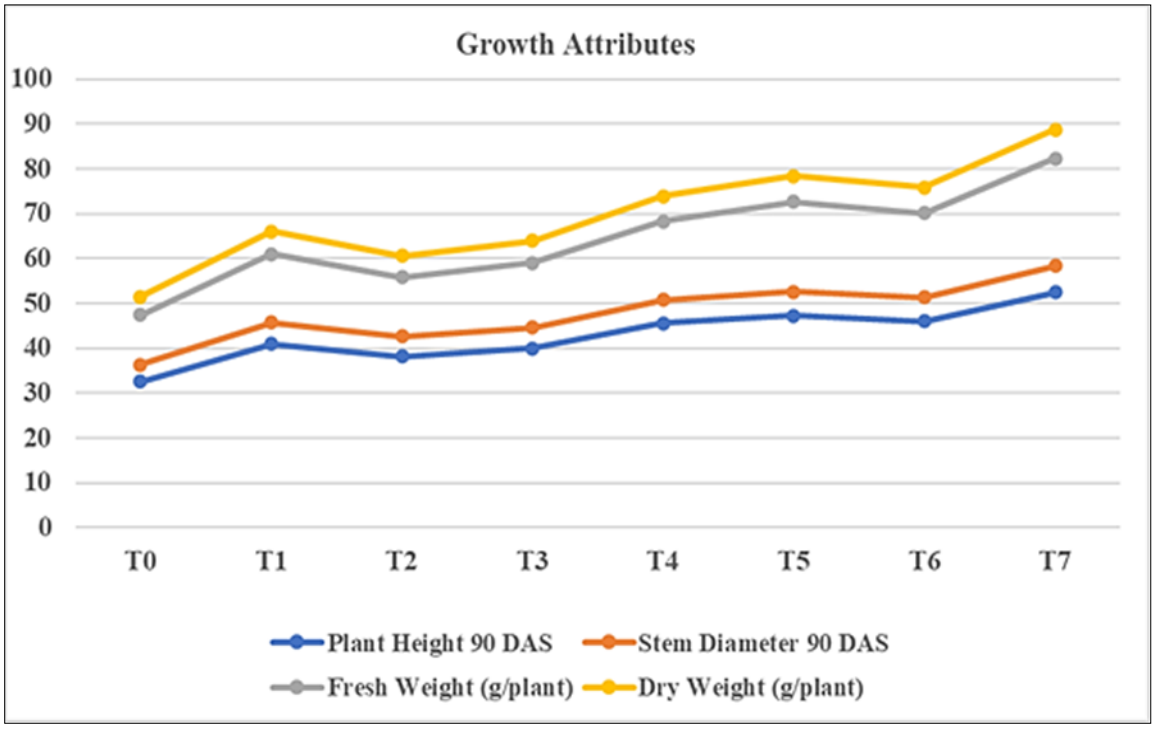

Fig. 6. Graph illustrating the growth parameter of *M. oleifera* plant.

Table 4. The nutrient uptake in *M. oleifera* plant

Treatment	Treatment code	% N Conc.	N uptake (mg/plant)	% P Conc.	P uptake (mg/plant)	% K Conc.	K uptake (mg/plant)
Control	T_0	1.35	53.46 ± 1.53	0.18	7.12 ± 0.203	1.08	42.76 ± 1.241
Azotobacter	T_1	1.47	74.08 ± 1.703	0.21	10.58 ± 0.231	1.20	60.48 ± 1.386
Pseudomonas	T_2	1.46	68.03 ± 2.367	0.20	9.32 ± 0.318	1.15	53.59 ± 1.848
Seaweed extract	T_3	1.50	73.65 ± 3.378	0.19	9.32 ± 0.433	1.13	55.48 ± 2.569
Azotobacter* + *Pseudomonas	T_4	1.66	93.12 ± 1.617	0.21	11.78 ± 0.203	1.29	72.36 ± 1.242
***Azotobacter* + Seaweed**	T_5	1.66	96.44 ± 2.223	0.21	12.20 ± 0.289	1.30	75.53 ± 1.761
***Pseudomonas* + Seaweed**	T_6	1.66	93.95 ± 2.685	0.21	11.88 ± 0.346	1.29	73.01 ± 2.107
***Azotobacter* + *Pseudomonas* + Seaweed**	T_7	1.76	113.69 ± 3.926	0.22	14.21 ± 0.491	1.38	89.14 ± 3.089
SEm (±)			2.643		0.341		2.066
CD (*P* = 0.05)			8.093		1.044		6.329

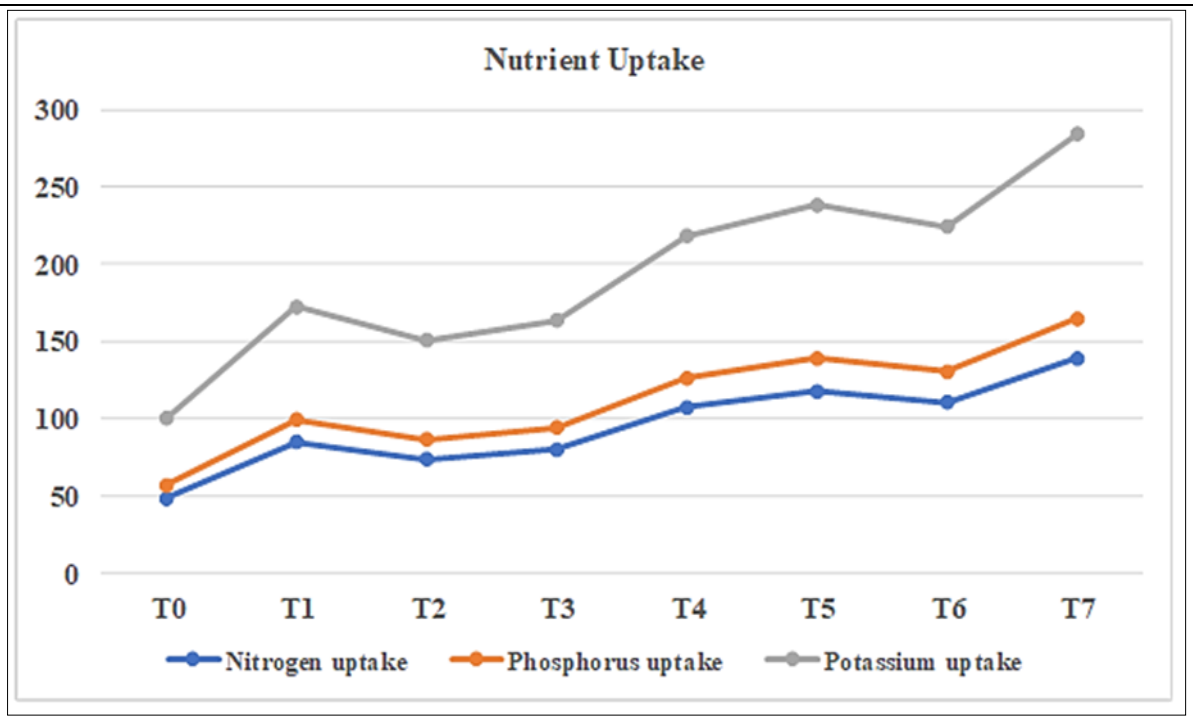

Fig. 7. Graph illustrating the nutrient uptake in *M. oleifera* plant.

Microbial population count of *Azotobacter*

The soil microbial analysis revealed that the combined application of *Azotobacter*, *Pseudomonas* and seaweed extract (T_7) recorded the highest *Azotobacter* population (9.397 ± 0.326 × 10^6 cfu/g), representing an 80.84 % increase over the control (1.8 × 10^6 cfu/g). Among dual treatments, T_5 (*Azotobacter* + seaweed extract) showed the next highest population (8.197±0.188 × 10^6 cfu/g), while the single inoculation with *Azotobacter* (T_1) yielded (6.897 ± 0.159 × 10^6 cfu/g). This enhanced microbial proliferation under combined treatments is attributed to the enhanced colonization potential and synergistic effect of bioinoculants and seaweed extract (Table 5 and Fig. 8).

Microbial population count of PSB

The population of phosphate-solubilizing bacteria (PSB) in soil varied significantly among treatments, highlighting the influence of microbial and bio stimulant combinations (Table 5 and Fig. 8). The highest PSB population (9.297 ± 0.32 × 10^5 cfu/g) was observed in the integrated treatment T_7 (*Azotobacter* + *Pseudomonas* + seaweed extract), followed by T_6 (*Pseudomonas* + seaweed extract) with (8.097 ± 0.234 × 10^5 cfu/g) and T_4 (*Azotobacter* + *Pseudomonas*) with (7.797 ± 0.136 × 10^5 cfu/g). In contrast, the control (T_0) recorded the lowest PSB count (2.097 ± 0.061 × 10^5 cfu/g).

Total soil microbial population count

The total soil microbial population in the soil rhizosphere varied significantly among treatments, demonstrating the beneficial impact of bioinoculants and seaweed extract on soil microbial population (Table 6 and Fig. 9). The integrated application of *Azotobacter*, *Pseudomonas* and seaweed extract (T_7) recorded the highest populations of bacteria (13.797 ± 0.476 × 10^5 cfu/g), actinomycetes (10.797 ± 0.372 × 10^6 cfu/g) and fungi (7.847 ± 0.274 × 10^4 cfu/g), significantly exceeding those of individual or dual treatments and control. Among dual applications, T_4 (*Azotobacter* + *Pseudomonas*) also showed substantial microbial enrichment, whereas the control (T_0) consistently exhibited the lowest counts across all groups.

Table 5. Microbial population count of *Azotobacter* and PSB

Treatment	Treatment code	*Azotobacter* (cfu/g × 10^6)	PSB (cfu/g × 10^5)
Control	T_0	1.8 ± 0.052	2.097 ± 0.061
Azotobacter	T_1	6.897 ± 0.159	2.397 ± 0.055
Pseudomonas	T_2	2.197 ± 0.078	6.697 ± 0.234
Seaweed extract	T_3	2.597 ± 0.118	3.197 ± 0.147
Azotobacter* + *Pseudomonas	T_4	7.597 ± 0.13	7.797 ± 0.136
***Azotobacter* + Seaweed**	T_5	8.197 ± 0.188	3.897 ± 0.09
***Pseudomonas* + Seaweed**	T_6	2.897 ± 0.084	8.097 ± 0.234
***Azotobacter* + *Pseudomonas* + Seaweed**	T_7	9.397 ± 0.326	9.297 ± 0.32
SEm (±)		0.161	0.18
CD (*P* = 0.05)		0.493	0.55

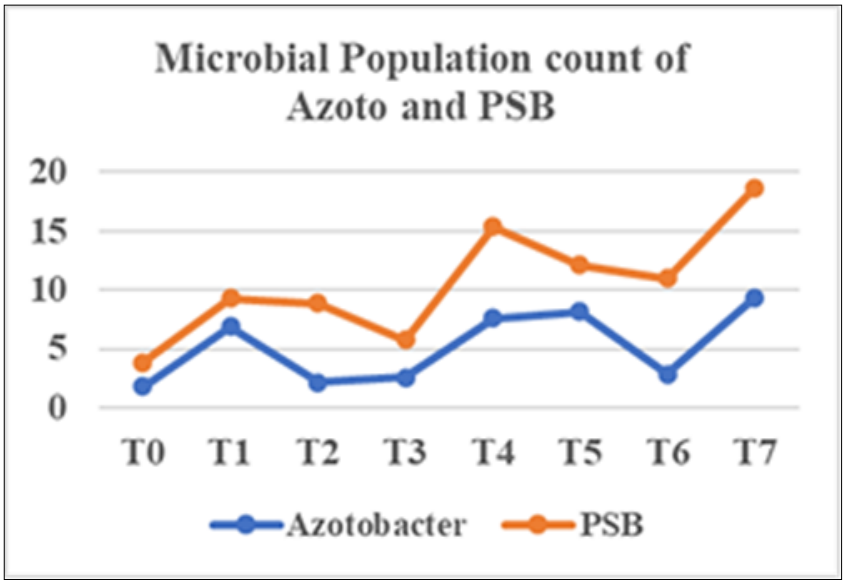

Fig. 8. Microbial count of *Azotobacter* and PSB.

Table 6. Total soil microbial population count after the interaction of bioinoculants

Treatment	Treatment code	Total soil microbial count		
		Bacteria (cfu/g × 10^5)	Actinomycetes (cfu/g × 10^6)	Fungi (cfu/g × 10^4)
Control	T_0	6.4 ± 0.185	7.297 ± 0.211	5.6 ± 0.162
Azotobacter	T_1	9.697 ± 0.222	8.597 ± 0.199	6.5 ± 0.15
Pseudomonas	T_2	8.797 ± 0.303	8.197 ± 0.286	6.097 ± 0.211
Seaweed extract	T_3	7.597 ± 0.349	7.797 ± 0.361	5.797 ± 0.268
Azotobacter* + *Pseudomonas	T_4	13.597 ± 0.234	10.697 ± 0.188	7.697 ± 0.136
Azotobacter* + *Seaweed	T_5	11.097 ± 0.257	9.597 ± 0.222	7.197 ± 0.165
Pseudomonas* + *Seaweed	T_6	10.8 ± 0.312	9.2 ± 0.266	6.897 ± 0.199
***Azotobacter* + *Pseudomonas* + Seaweed**	T_7	13.797 ± 0.476	10.797 ± 0.372	7.847 ± 0.274
SEm (±)		0.311	0.283	0.21
CD (*P* = 0.05)		0.953	0.866	0.644

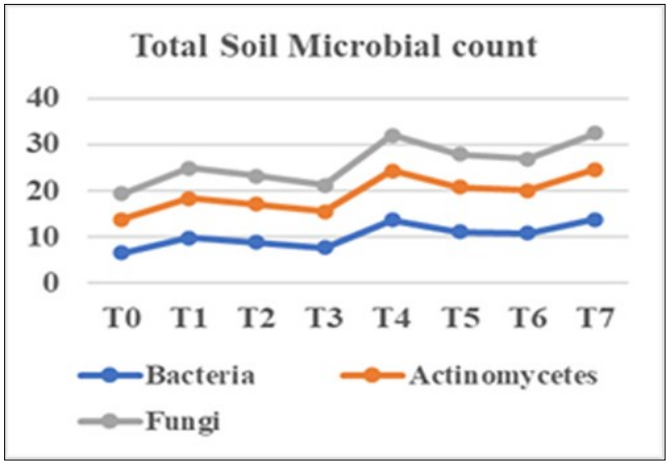

Fig. 9. Total soil microbial count.

Discussion

Effect on germination attributes of *M. oleifera* plant

The application of bioinoculants and bio stimulants markedly influenced the germination dynamics and early developmental traits of *M. oleifera*, with treatment-specific variations in efficacy (Fig. 10). Plants in the control group consistently exhibited inferior performance across key germination and vigour parameters. In contrast, treatments involving combinations of multiple microbial agents significantly outperformed single inoculations, corroborating earlier findings on microbial synergism in plant development (23, 24). Notably, the combined application of *Azotobacter*, *Pseudomonas* and seaweed extract yielded the most pronounced improvements, surpassing the effects of individual and dual combinations (25, 26). This outcome underscores the synergistic interaction among nitrogen-fixing bacteria, phosphate-solubilizing microbes and bioactive marine-derived compounds containing betaine and mannitol, collectively stimulating the plant mechanism, increases root-shoot vigour enhancing early physiological and metabolic processes (27). The findings affirm the potential of integrated microbial and bio stimulant strategies to optimize early-stage growth performance in *M. oleifera* cultivation (28).

Fig. 10. Germinated seedlings.

The enhanced germination and emergence under integrated treatments can be attributed to the complementary functional traits of the applied bio stimulants (Fig. 11). Nitrogen-fixing bacteria such as *Azotobacter* and phosphate-solubilizing microbes like *Pseudomonas,* classified as plant growth-promoting rhizobacteria (PGPR) enhance soil nutrient bioavailability and stimulate the synthesis of phytohormones such as indole-3-acetic acid (IAA), which collectively energize and enhance the root architecture and seedling vigour (23, 29). These microbial activities, when combined with the bioactive compounds in seaweed extracts

Fig. 11. Views of seedlings in portray and after transplanting in polybags.

such as polysaccharides, betaines and micronutrients enhance metabolic activation and facilitate early nutrient assimilation, thereby accelerating seedling establishment (19, 30, 31).

The reduced mean germination time (MGT) observed in the treated groups, especially in the triple-inoculant combination, suggests accelerated physiological responses during seed hydration and radicle emergence. This effect is likely due to enhanced enzymatic activation and nutrient mobilization facilitated by microbial consortia (21, 24, 32).

Enhanced germination indices such as speed of germination, mean germination rate, mean daily germination and peak value indicate not only improved germination rates but also uniformity, which is vital for successful field establishment. Concurrently, seaweed extracts contribute a rich matrix of organic compounds polysaccharides, vitamins and stress-alleviating betaines that facilitate enzymatic activation and seed metabolic priming (18, 33). This multidimensional approach enhances seed vigour, accelerates enzymatic activity involved in seed reserve mobilization and promotes more synchronized and rapid seedling emergence. These findings state the role of integrated microbial bio stimulant formulations in creating an optimal rhizospheric environment conducive to uniform and vigorous early plant growth.

The germination value (GV) and seedling vigour index (SVI) recorded under integrated treatments serve as strong indicators of early plant health and are predictive of overall crop performance and resilience (18, 21, 34). While single-agent treatments contributed positively, they couldn't match the synergistic force of the full combination, which delivered the most profound benefits across all early growth metrics. Seaweed extracts, particularly those derived from species like *Ascophyllum nodosum*, are rich in phytohormones including cytokinin, auxins and betaines, which enhance cell division, enzymatic activity and chlorophyll synthesis. These effects contribute to improved seedling vigour and germination efficiency, as confirmed in both current and recent studies (22, 35, 36).

Fig. 12. Comparison of *M. oleifera* seedlings growth under different treatments.

The superiority of triple combinations over dual and single treatments reinforces the significant advantage of multi-functional bioformulations in sustainable and effective approach for enhancing early-stage plant production. These findings align with emerging literature advocating for the integrated application of PGPR and bio stimulants to reduce chemical dependency while improving plant establishment under varied agroecological conditions (20).

Effect on growth attributes and nutrient uptake of *M. oleifera* plant

The present study evaluated the effect of various bio-inoculant and seaweed extract treatments on vegetative growth parameters and nutrient uptake (N, P, K) in plants at 90 days after sowing (DAS). The integration of biofertilizers, either alone or in combination, demonstrated a marked improvement in plant height, stem diameter, biomass accumulation and nutrient assimilation compared to the untreated control. The findings of this study demonstrate the significant role of microbial inoculants and bio stimulants in enhancing plant growth and nutrient uptake, particularly when applied in combination. The synergistic effects observed from the integration of *Azotobacter*, *Pseudomonas* and seaweed extract highlight the potential of such bio-based formulations in optimizing crop performance under sustainable cultivation practices.

The marked increase in plant height, stem diameter and biomass in the combined treatment (*Azotobacter* + *Pseudomonas* + Seaweed) may be attributed to the complementary mechanisms of action among the components (Fig. 12). *Azotobacter*, a well-documented nitrogen-fixing bacterium, enhances nitrogen availability and promotes root development through the secretion of phytohormones such as indole-3-acetic acid (IAA). *Pseudomonas* spp., known for their phosphate-solubilizing ability and siderophore production, contribute to improved phosphorus uptake and protection against soil-borne pathogens. Seaweed extract, rich in bioactive compounds including cytokinin, betaines and polysaccharides, further stimulates physiological and biochemical pathways involved in plant growth and stress resilience (37, 38).

The substantial increases in nitrogen, phosphorus and potassium uptake observed in dual and triple inoculant treatments are indicative of improved nutrient use efficiency. These enhancements are likely the result of both direct mechanisms such as nutrient solubilization and biological nitrogen fixation and indirect effects, including improved root architecture and increased microbial activity in the rhizosphere. The results align with previous studies reporting enhanced nutrient assimilation and biomass accumulation under integrated biofertilizer treatments (37, 38).

The application of single inoculants (*Azotobacter, Pseudomonas*) and seaweed extract individually led to moderate enhancements in all parameters, whereas their combinations (dual or triple) exhibited synergistic effects, significantly augmenting nutrient use efficiency and biomass accumulation. Interestingly, while single applications of *Azotobacter, Pseudomonas* or seaweed extract did yield positive effects over the control, their individual contributions were consistently surpassed by dual and triple combinations, emphasizing the advantage of multi-functional consortia. This synergy not only reinforces nutrient availability but also supports the idea of a more resilient soil-plant system.

These results underscore the potential of integrated biofertilizer strategies to improve plant growth and nutrient acquisition sustainably, thereby supporting their adoption as viable components in environmentally responsible agricultural practices. From a sustainability standpoint, the ability to reduce reliance on synthetic fertilizers through biologically active formulations offers significant agronomic and ecological benefits. The present study supports the paradigm shift toward environmentally responsible nutrient management practices, promoting both crop productivity and soil health.

Microbial population count of *Azotobacter*

The significantly higher *Azotobacter* population under the combined application of *Azotobacter*, *Pseudomonas* and seaweed extract (T_7) is primarily due to synergistic interactions that enhance rhizosphere suitability for microbial establishment. While *Azotobacter* contributes to nitrogen fixation, seaweed extract, rich in polysaccharides and organic stimulants provides easily assimilable carbon sources, amino acids and bioactive compounds that

stimulate root exudation and creates a favourable rhizospheric environment supporting microbial growth (18, 22, 33, 35). Concurrently, *Pseudomonas* spp. indirectly supports *Azotobacter* proliferation by improving nutrient availability through organic acid production and siderophore-mediated nutrient mobilisation (11, 29). The lower populations observed under single and dual treatments indicate that individual inputs alone are insufficient to maximise microbial persistence under soil nutrient constraints. These results confirms that co-application of functional microbes with bio stimulants significantly enhance soil microbial diversity, nutrient cycle and soil health and ultimately supports early development of *M. oleifera*.

Microbial population count of PSB

Thus, the higher population of phosphate-solubilizing bacteria recorded can be attributed to the superior performance of T_7 due to the combined effects of *Pseudomonas*-mediated phosphate solubilization with organic acid and siderophore production, this enhanced PSB population under combined treatments that attributed to the synergistic interactions between functional microbes and seaweed-derived compounds, while *Azotobacter* contributes nitrogen fixation enhancing the microbial persistence and root exudation patterns that support heterotrophic bacterial growth by the production of phytohormone (23, 29). Additionally, seaweed extract, rich in polysaccharides, vitamins and growth-promoting substances that easily assimilable carbon sources, amino acids along with bioactive compounds that stimulate and improves microbial habitat, its metabolism, cell division and supports enzyme-mediated nutrient cycling (22, 35, 39). The lower PSB populations observed under dual and single inoculations, together with the minimal density in the control, indicate that the absence of microbial interaction and bio stimulant inputs restricts nutrient cycling and limits sustained PSB proliferation under natural soil conditions.

Total soil microbial population count

Azotobacter and *Pseudomonas* contribute essential soil functions such as nitrogen fixation, phosphate solubilization and phytohormone excretion (23, 29), while seaweed extract enriches the microbial niche by polysaccharides, micronutrients and growth-regulating compounds (22, 39). Integrated formulations like T_7 have been shown to improve microbial biomass, enzyme activity and soil rhizospheric structure. The notably higher fungal population suggests that seaweed bio stimulants may extend their stimulatory effects beyond bacteria, enhancing microbial diversity and ecological stability.

Conclusion

In summary, the study demonstrates that the combined inoculation strategy application of treatment (T_7) *Azotobacter*, *Pseudomonas* and seaweed extract significantly improves germination efficiency, early growth performance and enhance the microbial community in the soil of *M. oleifera* than the individual and dual applications, suggesting a synergistic enhancement of physiological and metabolic processes essential for seedling development. Thus, the improvements suggest greater nutrient availability, balanced plant metabolism and active participation of beneficial soil microbes, that synergistically work to promote vigorous root and shoot growth during the early stages of plant establishment. These findings highlight the potential of microbial consortia and natural bio stimulants as sustainable alternatives to chemical inputs, eco-friendly by improving productivity and enhancing early plant establishment. Future research should aim to validate these outcomes under varied agroecological conditions and assess the long-term impact of bioinoculants on soil health, better quality planting material and enhanced productivity for nursery growers and foresters to support large-scale adoption.

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Authors' contributions

JP carried out the entire experiment and statistical analysis, SPM contributed to manuscript making and CP helped in understanding the recorded experimental parameters, CK helped in supporting manuscript making editing of the manuscript and SS, DNY contributed in arranging and facilitate requirements while the experiment was being carried out.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest

Ethical issues: None.

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