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RESEARCH ARTICLE

Biofertilizer pellets as a strategy to enhance soil nutrient dynamics and microbial populations in mulberry (*Morus indica* L.) cultivation

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Abstract

Organic pelleted fertilizers are vital for enhancing soil health and increasing microbial populations in mulberry (*Morus indica* L.) cultivation. They supply essential nutrients, improve soil structure, promote microbial activity and contributing to sustainable sericulture. This study evaluated the impact of pelleted biofertilizers on soil quality through a factorial pot experiment in a greenhouse using a randomized complete block design. Treatments included combinations of Orgafol, biofertilizers (*Azospirillum*, phosphobacteria, arbuscular mycorrhizal fungi-AMF) and growth promoters. Soil pH, electrical conductivity (EC), available N, P, K and culturable microbial populations were measured 90 days after planting. Orgafol + *Azospirillum* + AMF (T10) produced the highest EC (0.62 dS m^{-1}) and the lowest pH (6.48), whereas Orgafol alone maintained the highest pH (6.67). Nitrogen was maximised by Orgafol + naphthaleneacetic acid (NAA) + *Azospirillum* + AMF, phosphorus by Orgafol + phosphobacteria (13.32 kg ha^{-1}) and potassium by Orgafol + NAA + *Azospirillum* (228.98 kg ha^{-1}). Biofertilizer pellets markedly increased total microbial counts, peaking in Orgafol + NAA + *Azospirillum* + AMF + phosphobacteria (T9). PCA (principal component analysis) identified two principal components accounting for 73.27 % of the total variance. PC1 was strongly correlated with microbial counts and negatively with pH, while PC2 was associated with EC, phosphorus and potassium levels. Treatments T9 and T10 ranked highest in PC1 and T6 dominated PC2, highlighting their effectiveness in enhancing soil fertility. The study demonstrates that tailored pelleted biofertilizer blends can rapidly improve physicochemical properties and biological activity of mulberry soils, offering a scalable strategy for more sustainable and productive sericulture.

Keywords: AMF; *Azospirillum*; PCA; pelleted fertilizers; phosphobacteria; sericulture

Introduction

Soil, often perceived as static, is in fact a dynamic ecosystem teeming with biological activity and chemical processes (1). While the effects of biofertilizers on various crops are well established, their impact on mulberry, a key component in sericulture, remains relatively underexplored. The yield and quality of mulberry leaves, which are critical for silk production, are significantly influenced by factors such as soil type, plant variety, nutrient availability and environmental conditions (2). Regions with tropical to subtropical climates, such as southern India, including Karnataka and Tamil Nadu provide optimal conditions for mulberry cultivation due to their well-distributed rainfall, moderate temperatures and fertile soils. Traditionally, chemical fertilizers were the main nutrient source for mulberry cultivation; however, long term soil degradation has emphasized the need for more sustainable alternatives (2).

Recently, organic manures and biofertilizers enriched with beneficial microorganisms have gained attraction as environmentally friendly alternatives to chemical fertilizers (3, 4). These sustainable inputs are not only cost-effective but also improve soil characteristics and stimulate biological activity, which are vital for maintaining long-term soil fertility. Biofertilizers primarily consist of microorganisms that fix nitrogen, solubilise phosphate and promote plant growth, playing a significant role in enhancing the biological properties of soils (3). When used in combination with reduced doses of chemical fertilizers, organic amendments can greatly enhance soil fertility by increasing the availability of essential nutrients and boosting microbial populations (5).

Organic amendments support sustained crop production while enhancing soil microbial activity and diversity, essential for maintaining soil health and functionality (6). Microbial communities in the soil are involved in critical processes such as carbon and nutrient cycling, which are fundamental to sustainable agriculture (7). Moreover, higher microbial diversity is often linked to improved soil health and crop resilience, helping plants better withstand biotic and

abiotic stresses. Biofertilizers significantly improve soil chemical and microbial properties by enhancing nutrient availability, improving soil structure and promoting beneficial microbes such as nitrogen-fixers and phosphate solubilizers. They boost organic carbon, support nutrient cycling and increase the availability of key nutrients such as nitrogen and phosphorus, leading to better plant growth and productivity. Additionally, enhanced microbial activity also suppresses pathogens and strengthens plant resilience against environmental stresses (8).

Biofertilizers are formulations containing living microorganisms, available in solid or liquid carrier-based forms, that improve soil and plant health (9). Co-inoculation of microbial strains such as *Azospirillum* and *Rhizobium* spp. (nitrogen-fixing bacteria), *Bacillus* and *Pseudomonas* spp. (phosphate-solubilizing bacteria), along with arbuscular mycorrhizal fungi (AMF), has been shown to enhance plant growth, yield and soil microbial communities compared to single inoculants (9-11). This is largely due to the synergistic interactions among the microbes, which improve nutrient uptake efficiency, stimulate root development and enhance overall plant resilience. The effectiveness of biofertilizers can be further enhanced by providing appropriate nutrient sources to the microbes through carrier materials, making pelletized biofertilizers particularly valuable for their high quality and desirable properties (12-14).

Given this context, the present study aims to assess the impact of pelleted biofertilizers containing a consortium of beneficial microorganisms (*Azospirillum* spp. phosphobacteria and AMF) on mulberry soil health and microbial populations. The findings of this study will offer valuable insights for farmers, encouraging the adoption of sustainable and environmentally friendly practices in mulberry cultivation, ultimately enhancing silk production and soil health.

Materials and Methods

Preparation of bioinoculum

Azospirillum spp., phosphobacteria and AMF inoculum were used in biofertilizer production.

Azospirillum

Azospirillum spp. were isolated from the root samples of mulberry (*Morus* spp.) plant. The surface-sterilized root segments were then plated on semi-solid nitrogen-free bromothymol blue (Nfb) medium and incubated at 33 °C for 2-8 days (15). *Azospirillum* colonies appeared as subsurface white haloes originating from the cut ends of the roots and stems, eventually surrounding the entire root and stem segments. Typical colonies from the plates were streaked again on fresh Nfb semisolid medium for further confirmation and isolation of pure cultures as shown in Fig. 1 (15). Initially, *Azospirillum* spp. formed smooth, white or greyish colonies, which later became white and wrinkled. These pure cultures were then stored at 4 °C.

Phosphobacteria

Phosphobacteria species were isolated by serial dilution method from the randomly selected soil samples collected from undisturbed area of Forest College and Research Institute, Mettupalayam. Aliquots (0.1 mL) of 10^{-3} to 10^{-4} dilutions of the rhizosphere soil sample suspensions were inoculated on Pikovskaya's agar plates using pour plate technique, as shown in Fig. 2A and 2B and incubated at 28±2 °C for 4 days (16). PSB formed a halo zone around the colonies.

Arbuscular mycorrhizal fungi (AMF)

To prepare the inoculums of indigenous AMF, rhizosphere soil sample were collected from rhizosphere region of mulberry and were filled in sterile polythene bags. The extraction of AMF spores was conducted using the wet sieving and decanting method (17). The starter inoculum of the selected AMF was raised using the funnel technique using onion as a hostplant (18). Root fragments of onion together with rhizosphere soil are considered as an AMF inoculum.

Preparation of organic growth promoter pellets

The pure cultures of isolated bacteria were again cultured in a nutrient-rich medium comprising of yeast extract (20 g L^{-1}), beef extract (20 g L^{-1}), peptone (20 g L^{-1}), finely ground bone meal powder (20 g L^{-1}) and agar (1 g L^{-1}). For emulsification, 250 mL of water was boiled and added to 50 g of molten beeswax, followed by 2 g of borax and heated until fully dissolved. This mixture was then incorporated at 100 ml L^{-1} into the nutrient medium. For pelletization, lignite and guar gum were combined as carrier and binder in the ratio of 39:1, mixed with an organic growth promoter and processed through a pelletizer to form pellets as shown in Fig. 3.

Study area

A pot experiment was conducted in a greenhouse under natural light conditions at the Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam. This location is situated at 11.20° North latitude and 76.56° East longitude, with an elevation of 320 meters above mean sea level. During the experiment, the greenhouse temperature ranged from 31-42 °C, with a relative humidity of 68 %. Cuttings from V1 mulberry (*M. indica* L.) variety was used as the planting material. Before the start of the experiment, the soil parameters were measured and recorded as follows: pH 6.97, EC 0.38 dS m^{-1}, available nitrogen 188.06 kg ha^{-1}, available phosphorus 10.27 kg ha^{-1} and available potassium 215 kg ha^{-1}.

Treatments and experimental design

Fig. 1. Colonies of *Azospirillum* spp. on nitrogen free bromothymol blue nitrogen free bromothymol blue (Nfb) medium cultured using the streak plate technique.

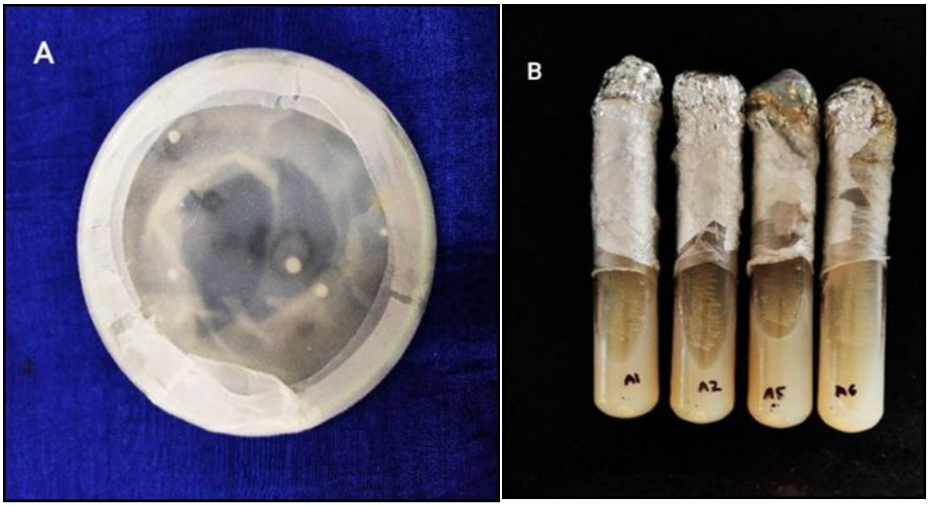

Fig. 2. Cultured phosphobacteria species on Pikovskaya's medium using (A) pour plate technique, (B) streak plate technique.

Fig. 3. Pelletized biofertilizers prepared from *Azospirillum*, phosphobacteria and AMF cultures.

Treatment details

The pelleted biofertilizer was then applied in five different concentrations: 4 g (P1), 5 g (P2), 10 g (P3), 15 g (P4) and 20 g (P5) pellet per plant as shown in Table 1.

Experimental design

The study utilized a factorial design with a randomized complete block structure, including four replications. The experiment involved two factors: the type of pellet fertilizer and the concentration of fertilizer applied.

Soil sampling and analysis

Table 1. Summary of the experimental treatments

Treatment No.	Treatment compositions
T1	Orgafol
T2	Orgafol + NAA
T3	Orgafol + *Azospirillum*
T4	Orgafol + Phosphobacteria
T5	Orgafol + AMF
T6	Orgafol + NAA + *Azospirillum*
T7	Orgafol + NAA + Phosphobacteria
T8	Orgafol + NAA + AMF
T9	Orgafol + NAA + *Azospirillum* + Phosphobacteria
T10	Orgafol + *Azospirillum* + AMF

NAA- Naphthalene acetic acid; AMF- Arbuscular mycorrhizal fungi

The research was conducted over a period of three months. At the conclusion of the experiment, soil parameters such as pH, electrical conductivity (EC), microbial population (log cfu g^{-1}) and the content of available nitrogen, phosphorus and potassium were recorded. The pH and EC of the collected soil samples were determined using the potentiometry method and an EC meter, respectively (19). The available nitrogen, phosphorus and potassium content was estimated using the alkaline permanganate method, Olsen method and neutral normal ammonium acetate method, respectively (20-22).

Statistical analysis

The data collected were analyzed using OPSTAT and SPSS 23 software at a 5 % probability level and the PCA was carried out using XLSTAT 24 software.

Results

Electrical conductivity (EC)

Table 2 shows the results of the study which indicate significant ($p< 0.05$) variations in EC across different 10 treatments with 5 concentrations. Treatment T10 exhibited the highest EC values, ranging from 0.58 to 0.62 dS m^{-1}. In contrast, treatment T8 had the lowest EC values, ranging from 0.27 to 0.42 dS m^{-1}. The overall mean EC values for the concentrations increased from 0.43 to 0.49 dS m^{-1} as the concentration increased from the lowest to the highest level.

Soil reaction (pH)

Table 3 illustrate the impact of various treatments on soil pH. Significant differences in pH levels were observed across treatments and concentrations ($p < 0.05$). Treatment T1 recorded the highest pH values, ranging from 6.62 to 6.67, treatment T10 showed the lowest pH values, ranging from 6.48 to 6.52. Overall, the mean pH increased from 6.56 to 6.60 as the concentration increased from the lowest to the highest level.

Available soil nitrogen

Table 4 presents the concentrations of available soil nitrogen across various treatments and application rates of organic pellet fertilizers ranging from 4 g to 20 g per plant. Treatment T9 (Orgafol + NAA + *Azospirillum* + phosphobacteria) consistently showed the highest nitrogen content, reaching 220.63 kg ha^{-1}at

Table 2. Electrical conductivity (d Sm^{-1}) of soil samples across different treatments

Treatments	Concentrations				
	4 g pellet/ plant	5 g pellet/ plant	10 g pellet/ plant	15 g pellet/ plant	20 g pellet/ plant
T1 (Orgafol)	0.50 ± 0.009^{c}	0.52 ± 0.005^{c}	0.53 ± 0.005^{c}	0.54 ± 0.005^{c}	0.55 ± 0.006^{c}
T2 (Orgafol + NAA)	0.40 ± 0.008^{g}	0.42 ± 0.005^{f}	0.43 ± 0.003^{g}	0.44 ± 0.006^{g}	0.44 ± 0.008^{g}
T3 (Orgafol + *Azospirillum)*	0.38 ± 0.004^{h}	0.39 ± 0.004^{g}	0.41 ± 0.004^{h}	0.42 ± 0.004^{h}	0.43 ± 0.004^{gh}
T4 (Orgafol + Phosphobacteria)	0.53 ± 0.005^{b}	0.55± 0.005^{b}	0.56 ± 0.003^{b}	0.57 ± 0.006^{b}	0.58 ± 0.005^{b}
T5 (Orgafol + AMF)	0.33 ± 0.006^{i}	0.34 ± 0.004^{h}	0.35 ± 0.004^{i}	0.36 ± 0.005^{i}	0.37 ± 0.005^{i}
T6 (Orgafol + NAA + *Azospirillum)*	0.44 ± 0.004^{e}	0.45 ± 0.004^{e}	0.46 ± 0.004^{e}	0.47 ± 0.004^{e}	0.48 ± 0.004^{e}
T7 (Orgafol + NAA + Phosphobacteria)	0.46 ± 0.004^{d}	0.48 ± 0.006^{d}	0.48 ± 0.005^{d}	0.49 ± 0.004^{d}	0.50 ± 0.005^{d}
T8 (Orgafol + NAA + AMF)	0.27 ± 0.004^{j}	0.28 ± 0.004^{i}	0.29 ± 0.006^{j}	0.31 ± 0.007^{j}	0.42 ± 0.005^{h}
T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)	0.42 ± 0.004^{f}	0.43 ± 0.004^{f}	0.44 ± 0.006^{f}	0.45 ± 0.004^{f}	0.46 ± 0.004^{f}
T10 (Orgafol + *Azospirillum* + AMF)	0.58 ± 0.006^{a}	0.59 ± 0.006^{a}	0.59 ± 0.006^{a}	0.61 ± 0.004^{a}	0.62 ± 0.004^{a}
Mean	0.43	0.45	0.45	0.47	0.49

The average values obtained from four replications and the results were expressed as mean ± S.E. Means in a similar row with different letters are statistically significant at p <0.05 and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant.

Table 3. Soil reaction (pH) of soil samples across different treatments

Treatments	Concentrations				
	4 g pellet/ plant	5 g pellet/ plant	10 g pellet/ plant	15 g pellet/ plant	20 g pellet/ plant
T1 (Orgafol)	6.62 ± 0.004^{a}	6.63 ± 0.004^{a}	6.64 ± 0.004^{a}	6.66 ± 0.007^{a}	6.67 ± 0.005^{a}
T2 (Orgafol + NAA)	6.57 ± 0.004^{c}	6.58 ± 0.004^{c}	6.59 ± 0.006^{c}	6.60 ± 0.009^{c}	6.61 ± 0.004^{d}
T3 (Orgafol + *Azospirillum)*	6.61 ± 0.004^{a}	6.63 ± 0.006^{a}	6.64 ± 0.006^{a}	6.64 ± 0.005^{a}	6.65 ± 0.009^{b}
T4 (Orgafol + Phosphobacteria)	6.55 ± 0.004^{d}	6.56 ± 0.004^{d}	6.57 ± 0.004^{d}	6.58 ± 0.005^{d}	6.59 ± 0.008^{e}
T5 (Orgafol + AMF)	6.53 ± 0.004^{e}	6.54 ± 0.004^{e}	6.56 ± 0.006^{e}	6.57 ± 0.005^{de}	6.57 ± 0.007^{f}
T6 (Orgafol + NAA + *Azospirillum)*	6.59 ± 0.007^{b}	6.60 ± 0.008^{b}	6.61 ± 0.009^{b}	6.62 ± 0.010^{bc}	6.62 ± 0.004^{d}
T7 (Orgafol + NAA + Phosphobacteria)	6.58 ± 0.004^{bc}	6.59 ± 0.004^{c}	6.60 ± 0.005^{bc}	6.62 ± 0.005^{b}	6.63 ± 0.005^{c}
T8 (Orgafol + NAA + AMF)	6.53 ± 0.006^{e}	6.54 ± 0.004^{e}	6.54 ± 0.009^{f}	6.56 ± 0.006^{e}	6.56 ± 0.005^{f}
T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)	6.50 ± 0.005^{f}	6.52 ± 0.006^{f}	6.53 ± 0.005^{g}	6.54 ± 0.003^{f}	6.54 ± 0.007^{g}
T10 (Orgafol + *Azospirillum* + AMF)	6.48 ± 0.004^{g}	6.49 ± 0.004^{g}	6.50 ± 0.005^{h}	6.51 ± 0.004^{g}	6.52 ± 0.004^{h}
Mean	6.56	6.57	6.58	6.59	6.60

The average values obtained from four replications and the results were expressed as mean ± S.E. Means in a similar row with different letters are statistically significant at p<0.05 and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant.

20 g per plant, indicating the most effective combination for enhancing soil nitrogen. Conversely, treatment T4 (Orgafol + phosphobacteria) recorded the lowest nitrogen content across all concentrations, with values around 161.15 kg ha^{-1} at the highest application rate. The data suggest that treatments combining multiple bio-enhancers, particularly T9, significantly boost soil nitrogen availability compared to single-component treatments like T1 (Orgafol) or T3 (Orgafol + *Azospirillum*). Additionally, incremental increases in pellet concentrations resulted in marginal yet consistent rises in nitrogen content across all treatments, highlighting the importance of both the type and concentration of fertilizers in optimizing soil nutrient levels.

Available soil phosphorus

Table 5 presents the concentrations of available soil phosphorus across various treatments and application rates of organic pellet fertilizers from 4 g to 20 g per plant. Treatment T4 (Orgafol + phosphobacteria) consistently showed the highest phosphorus levels, reaching 13.32 kg ha^{-1} at the highest concentration of 20 g per plant. In contrast, the lowest phosphorus concentration was observed in treatment T8 (Orgafol + NAA + AMF), with a value of 10.18 kg ha^{-1} at 20 g per plant. Across all treatments, increasing the pellet concentration from 4 g to 20 g per plant resulted in slight but consistent increases in soil phosphorus levels. The data indicate that treatments incorporating phosphobacteria, particularly T4 and T9 (Orgafol + NAA + *Azospirillum* + phosphobacteria), were the most effective in enhancing soil phosphorus availability compared to other combinations.

Available soil potassium

Table 6 shows the effects of various treatments and concentrations of organic pellet fertilizers on available soil potassium levels. Among all treatments, T6 (Orgafol + NAA + *Azospirillum*) exhibited the highest potassium concentration, reaching 228.98 kg ha^{-1} at 20 g per plant, indicating its superior efficacy in enhancing soil potassium. On the other hand, the lowest potassium levels were observed in treatment T8 (Orgafol + NAA + AMF), with a concentration of 210.25 kg ha^{-1} at the highest application rate. Overall, increasing the pellet concentration from 4 g to 20 g per plant resulted in consistent but minor increases in soil potassium across all treatments.

Microbial population

The application of organic pellet fertilizer has a positive impact on the population of bacteria, fungi and actinomycetes in the soil, promoting microbial activity and contributing to improved soil health. The study revealed that the effects varied with treatment type and concentration. The population of bacteria in the soil, measured in log cfu g^{-1}, showed a marked increase with the application of organic pellet fertilizer (Fig. 4). The treatment T1P1 (Orgafol at 4 g pellet per plant) recorded the lowest bacterial count at 1.1475 log cfu g^{-1}, whereas the highest count was found in treatment T9P5 (Orgafol + NAA + *Azospirillum* + phosphobacteria at 20 g pellet per plant), with a population of 1.90 log cfu g^{-1}. These results suggest that higher concentrations of organic pellet fertilizer promote a significant increase in bacterial populations, with treatment T9 reaching the peak density.

Table 4. Available soil nitrogen concentrations (kg ha^{-1}) across different treatments

Treatments	Concentrations				
	4 g pellet/ plant	5 g pellet/ plant	10 g pellet/ plant	15 g pellet/ plant	20 g pellet/ plant
T1 (Orgafol)	210.18 ± 0.024^{c}	210.19 ± 0.001^{c}	210.20 ± 0.024^{c}	210.21 ± 0.001^{c}	210.22 ± 0.004^{c}
T2 (Orgafol + NAA)	198.80 ± 2.846^{e}	198.81 ± 2.843^{e}	198.82 ± 2.845^{e}	198.83 ± 2.846^{e}	198.84 ± 2.845^{e}
T3 (Orgafol + *Azospirillum)*	189.80 ± 2.596^{f}	189.81 ± 2.594^{f}	189.82 ± 2.597^{f}	189.83 ± 2.601^{f}	189.84 ± 2.598^{f}
T4 (Orgafol + Phosphobacteria)	161.11 ± 7.175^{j}	161.12 ± 7.168^{j}	161.13 ± 7.172^{j}	161.14 ± 7.172^{j}	161.15 ± 7.171^{j}
T5 (Orgafol + AMF)	172.88 ± 0.004^{h}	172.89 ± 0.001^{h}	172.90 ± 0.001^{h}	172.91 ± 0.006^{h}	172.92 ± 0.020^{h}
T6 (Orgafol + NAA + *Azospirillum)*	216.06 ± 0.001^{b}	216.08 ± 0.037^{b}	216.08 ± 0.021^{b}	216.09 ± 0.001^{b}	216.11 ± 0.001^{b}
T7 (Orgafol + NAA + Phosphobacteria)	163.27 ± 10.796^{i}	163.28 ± 10.797^{i}	163.29 ± 10.795^{i}	163.30 ± 10.796^{i}	163.31 ± 10.797^{i}
T8 (Orgafol + NAA + AMF)	187.27 ± 15.238^{g}	187.28 ± 15.237^{g}	187.29 ± 15.241^{g}	187.30 ± 15.238^{g}	187.31 ± 15.240^{g}
T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)	220.59 ± 5.997^{a}	220.60 ± 5.998^{a}	220.61 ± 5.999^{a}	220.62 ± 5.999^{a}	220.63 ± 6.001^{a}
T10 (Orgafol + *Azospirillum* + AMF)	202.85 ± 0.001^{d}	202.86 ± 0.006^{d}	202.87 ± 0.022^{d}	202.88 ± 0.031^{d}	202.89 ± 0.006^{d}
Mean	192.28	192.29	192.30	192.31	192.32

The average values obtained from four replications and the results were expressed as mean ± S.E. Means in a similar row with different letters are statistically significant at p<0.05 and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant.

Table 5. Available soil phosphorus concentrations (kg ha^{-1}) across different treatments

Treatments	Concentrations				
	4 g pellet/ plant	5 g pellet/ plant	10 g pellet/ plant	15 g pellet/ plant	20 g pellet/ plant
T1 (Orgafol)	13.02 ± 0.004^{c}	13.03 ± 0.004^{c}	13.04 ± 0.005^{c}	13.05 ± 0.004^{c}	13.06 ± 0.004^{c}
T2 (Orgafol + NAA)	12.82 ± 0.006^{d}	12.83 ± 0.001^{d}	12.84 ± 0.005^{d}	12.85 ± 0.004^{d}	12.86 ± 0.003^{d}
T3 (Orgafol + *Azospirillum)*	11.15± 0.005^{g}	11.16 ± 0.005^{h}	11.17 ± 0.003^{h}	11.18 ± 0.004^{g}	11.19 ± 0.004^{g}
T4 (Orgafol + Phosphobacteria)	13.28 ± 0.004^{a}	13.29 ± 0.004^{a}	13.30 ± 0.006^{a}	13.31 ± 0.004^{a}	13.32 ± 0.004^{a}
T5 (Orgafol + AMF)	10.26 ± 0.004^{h}	10.27 ± 0.004^{i}	10.28 ± 0.004^{i}	10.29 ± 0.004^{h}	10.30 ± 0.005^{h}
T6 (Orgafol + NAA + *Azospirillum)*	12.57 ± 0.004^{e}	12.58 ± 0.004^{e}	12.59 ± 0.004^{e}	12.60 ± 0.004^{e}	12.62 ± 0.007^{e}
T7 (Orgafol + NAA + Phosphobacteria)	12.55 ± 0.004^{f}	12.56 ± 0.004^{f}	12.57 ± 0.002^{f}	12.58 ± 0.004^{f}	12.59 ± 0.004^{f}
T8 (Orgafol + NAA + AMF)	10.14 ± 0.006^{i}	10.15 ± 0.005^{j}	10.17 ± 0.006^{j}	10.17 ± 0.005^{i}	10.18 ± 0.004^{i}
T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)	13.24 ± 0.004^{b}	13.25 ± 0.004^{b}	13.26 ± 0.004^{b}	13.27 ± 0.007^{b}	13.28 ± 0.004^{b}
T10 (Orgafol + *Azospirillum* + AMF)	11.16 ± 0.004^{g}	11.17 ± 0.004^{g}	11.18 ± 0.005^{g}	11.19 ± 0.004^{g}	11.20 ± 0.004^{g}
Mean	12.02	12.03	12.04	12.05	12.06

The average values obtained from four replications and the results were expressed as mean ± S.E. Means in a similar row with different letters are statistically significant at p<0.05 and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant.

Table 6. Influence of treatments on available soil potassium (kg ha^{-1})

Treatments	Concentrations				
	4 g pellet/ plant	5 g pellet/ plant	10 g pellet/ plant	15 g pellet/ plant	20 g pellet/ plant
T1 (Orgafol)	223.37 ± 0.034^{c}	223.38 ± 0.001^{c}	223.39 ± 0.001^{c}	223.40 ± 0.024^{c}	223.41 ± 0.034^{c}
T2 (Orgafol + NAA)	223.32 ± 0.034^{d}	223.33 ± 0.004^{d}	223.34 ± 0.034^{d}	223.35 ± 0.001^{d}	223.36 ± 0.001^{d}
T3 (Orgafol + *Azospirillum)*	222.47 ± 0.001^{g}	222.48 ± 0.024^{g}	222.49 ± 0.041^{h}	222.50 ± 0.001^{g}	222.51 ± 0.024^{g}
T4 (Orgafol + Phosphobacteria)	223.11 ± 0.001^{f}	223.12 ± 0.024^{f}	223.13 ± 0.001^{g}	223.14 ± 0.042^{f}	223.15 ± 0.024^{f}
T5 (Orgafol + AMF)	215.10 ± 0.003^{h}	215.11 ± 0.001^{h}	215.12 ± 0.024^{i}	215.13 ± 0.001^{h}	215.14 ± 0.004^{h}
T6 (Orgafol + NAA + *Azospirillum)*	228.94 ± 0.001^{a}	228.95 ± 0.025^{a}	228.96 ± 0.001^{a}	228.97 ± 0.001^{a}	228.98 ± 0.024^{a}
T7 (Orgafol + NAA + Phosphobacteria)	223.12 ± 0.024^{f}	223.13 ± 0.001^{f}	223.14 ± 0.001^{f}	223.15 ± 0.001^{f}	223.16 ± 0.042^{f}
T8 (Orgafol + NAA + AMF)	210.22 ± 0.024^{i}	210.22 ± 0.001^{i}	210.23 ± 0.003^{j}	210.24 ± 0.040^{i}	210.25 ± 0.004^{i}
T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)	223.17 ± 0.004^{e}	223.18 ± 0.024^{e}	223.19 ± 0.001^{e}	223.20 ± 0.024^{e}	223.21 ± 0.001^{e}
T10 (Orgafol + *Azospirillum* + AMF)	225.08 ± 0.004^{b}	225.09 ± 0.042^{b}	225.10 ± 0.001^{b}	225.11 ± 0.048^{b}	225.12 ± 0.024^{b}
Mean	221.79	221.80	221.81	221.82	221.83

The average values obtained from four replications and the results were expressed as mean ± S.E. Means in a similar row with different letters are statistically significant at p <0.05 and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant.

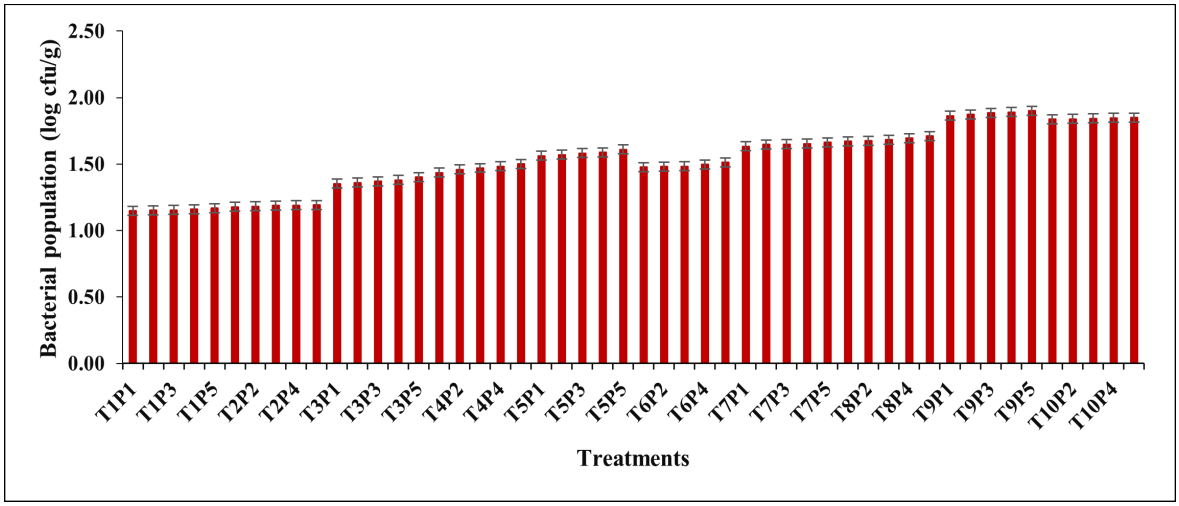

Fig. 4. Bacterial population (log cfu/g) across different treatments.

Ten treatments: T1=Orgafol, T2= Orgafol + NAA, T3= Orgafol + *Azospirillum,* T4= Orgafol + phosphobacteria, T5= Orgafol + AMF, T6= Orgafol + NAA + *Azospirillum,* T7= Orgafol + NAA + phosphobacteria, T8= Orgafol + NAA + AMF, T9= Orgafol + NAA + *Azospirillum* + phosphobacteria, T10= Orgafol + *Azospirillum* + AMF at five different concentrations P1= 4 g pellet/ plant, P2= 5 g pellet/ plant, P3= 10 g pellet/ plant, P4= 15 g pellet/ plant, P5= 20 g pellet/ plant. The analysis is carried out according to factorial randomised block design for two factors.

Similarly, the fungal population in soil also responded positively to the application of organic pellet fertilizer as demonstrated in Fig. 5. The treatment T1P1(Orgafol at 4 g pellet per plant) had the lowest fungal population of 0.55 log cfu g^{-1}. In contrast, treatment T9P5 (Orgafol + NAA + *Azospirillum* + phosphobacteria at 20 g pellet per plant) exhibited the highest population of fungi, reaching 1.59 log cfu g^{-1}. The overall trend shows that higher concentrations of organic pellet fertilizer correlate with increased fungal populations, with treatment T9 showing the most substantial growth.

The influence of organic pellet fertilizer on actinomycetes populations was also significant as shown in Fig. 6. The treatment T1P1(Orgafol at 4 g pellet per plant) had the lowest population of 1.65 log cfu g^{-1}. Treatment T9P5 (Orgafol + NAA + *Azospirillum* + phosphobacteria at 20 g pellet per plant) again recorded the highest population density with a mean of 2.25 log cfu g^{-1}. The data demonstrates that actinomycetes populations are highly responsive to the application of organic pellet fertilizer, particularly at higher concentrations, as evidenced by the results from treatment T9.

Principal component analysis (PCA)

Principal component analysis (PCA) is a dimensionality reduction technique that condenses a large set of variables into a smaller subset that captures most of the variance within the original data matrix. In this study, PCA was applied to assess the impact of pelleted biofertilizer treatments on soil chemical properties (EC, pH) and soil microbial populations. As shown in Table 7, the analysis identified two principal components (PCs) with the Eigenvalues greater than 1.0 out of eight, collectively explaining approximately 73.265 % of the total variability among the treatments.

The first principal component (PC1) accounted for 40.37 % of the variance and was strongly positively correlated with bacterial (0.967), fungal (0.943) and actinomycetes (0.897) counts in the treated soil. Conversely, PC1 showed a strong negative correlation with soil pH (-0.746) as shown in Fig. 7. The second principal component (PC2) explained 32.895 % of the variance and was positively associated with potassium content (0.938), phosphorus content (0.858) and EC (0.778). In contrast, PC2 exhibited a negative relationship with bacterial count (-0.192).

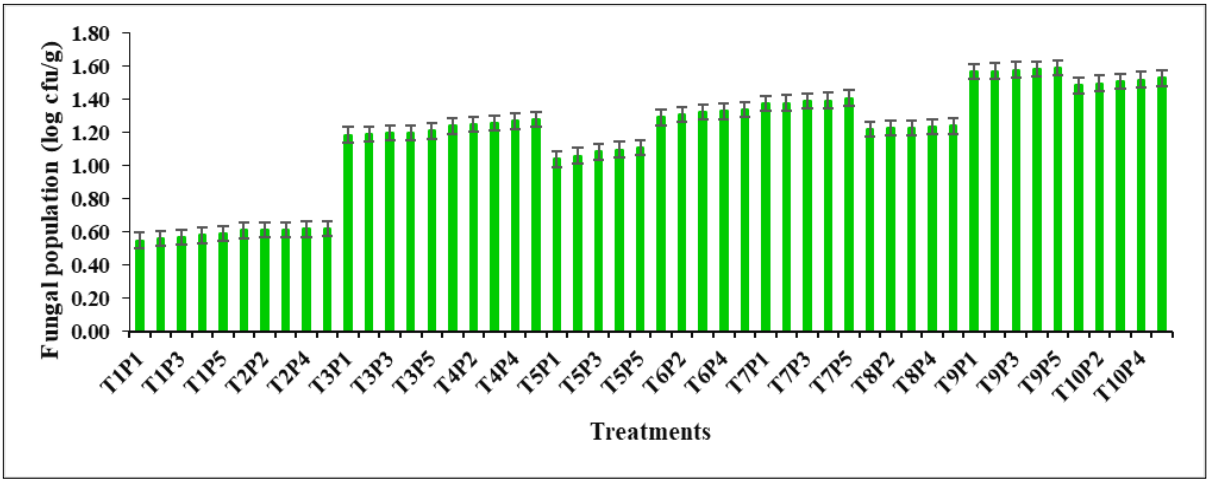

Fig. 5. Fungal population (log cfu/g) across different treatments.

Ten treatments: T1=Orgafol, T2= Orgafol + NAA, T3= Orgafol + *Azospirillum,* T4= Orgafol + phosphobacteria, T5= Orgafol + AMF, T6= Orgafol + NAA + *Azospirillum,* T7= Orgafol + NAA + phosphobacteria, T8= Orgafol + NAA + AMF, T9= Orgafol + NAA + *Azospirillum* + phosphobacteria, T10= Orgafol + *Azospirillum* + AMF at five different concentrations P1= 4 g pellet/ plant, P2= 5 g pellet/ plant, P3= 10 g pellet/ plant, P4= 15 g pellet/ plant, P5= 20 g pellet/ plant. The analysis is carried out according to factorial randomised block design for two factors.

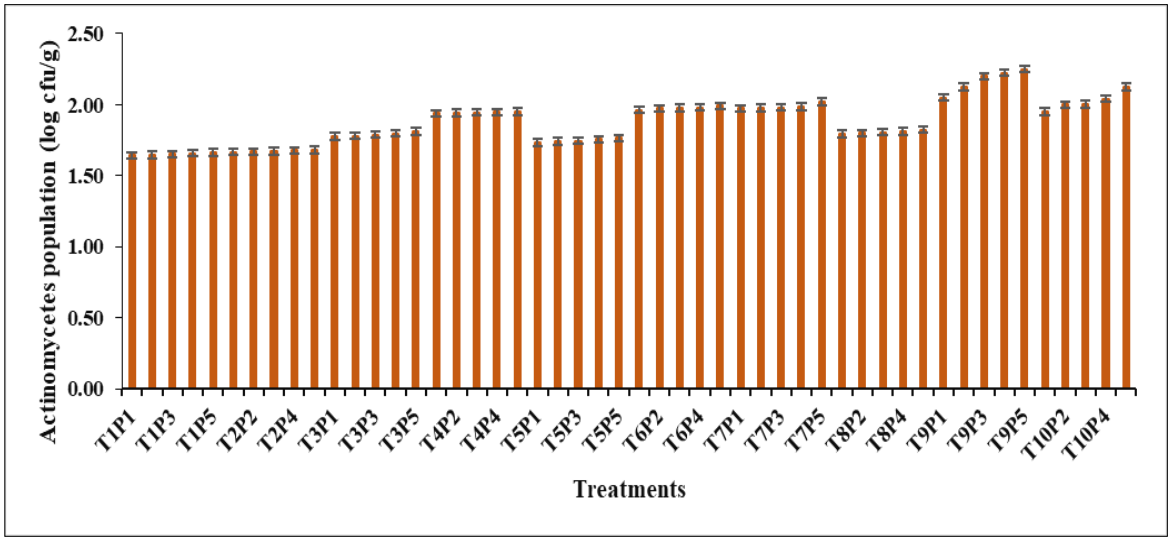

Fig. 6. Actinomycetes population (log cfu/g) across different treatments.

Ten treatments: T1=Orgafol, T2= Orgafol + NAA, T3= Orgafol + *Azospirillum,* T4= Orgafol + phosphobacteria, T5= Orgafol + AMF, T6= Orgafol + NAA + *Azospirillum,* T7= Orgafol + NAA + phosphobacteria, T8= Orgafol + NAA + AMF, T9= Orgafol + NAA + *Azospirillum* + phosphobacteria, T10= Orgafol + *Azospirillum* + AMF at five different concentrations P1= 4 g pellet/ plant, P2= 5 g pellet/ plant, P3= 10 g pellet/ plant, P4= 15 g pellet/ plant, P5= 20 g pellet/ plant. The analysis is carried out according to factorial randomised block design for two factors.

Based on principal component scores, treatments with positive values exceeding 1.0 were selected for both principal components (PC1 and PC2). For PC1, positive scores ranged from 2.967 (T9P5) to 2.491 (T10P1), while for PC2, positive scores ranged from 1.851 (T6P5) to 1.001 (T4P3) as shown in Table 8. Treatments associated with PC1 exhibited high positive values, indicating their significant contribution to the primary principal component, which accounts for the greatest variance in the dataset. Similarly, treatments associated with PC2 demonstrated high positive values, reflecting their strong influence on the secondary principal component.

The highest scores in PC1 were observed in treatments T9P5, T9P4, T9P3, T10P5 and T9P2, highlighting their strong association with the primary variance captured by PC1. In contrast, the top scores in PC2 were attributed to treatments T6P5, T6P4, T6P3, T1P5 and T6P2, underscoring their substantial influence on the secondary variance component. T9P5 ranked highest in PC1, indicating its dominant impact on the variance associated with this component, followed by T9P4, T9P3, T10P5 and T9P2. Similarly, T6P5 was the leading treatment in PC2, demonstrating its significant role in explaining the secondary variance, followed by T6P4, T6P3, T1P5 and T6P2.

Discussion

Soil pH is a key factor influencing nutrient availability, microbial activity and overall soil health. Nutrient solubility is optimal in a near-neutral pH range (6.0-7.0), where most essential nutrients are readily accessible (23). Extreme pH conditions, whether too acidic or too alkaline, can reduce nutrient availability and disrupt microbial functions, which can adversely affect plant growth. Effective management of soil pH helps create a balanced environment that supports nutrient uptake, healthy microbial communities and enhanced soil productivity. In this study, treatment T1 (Orgafol) had the highest soil pH values, while T10 (Orgafol + *Azospirillum* + AMF) had the lowest pH values. The decrease in soil pH observed in T10 could be attributed to the release of organic acids by AM fungi (24).

Nitrogen, phosphorus and potassium are essential macronutrients critical for plant growth and soil health. Nitrogen supports protein and chlorophyll synthesis, influencing plant vigour, leaf growth and overall yield, while also promoting soil microbial activity and nutrient cycling. Phosphorus plays a key role in energy transfer, root development and flowering, enhancing plant resilience and productivity, as well as supporting microbial processes vital for nutrient cycling. Potassium regulates water uptake, enzyme activation and stress tolerance, contributing to disease resistance, drought tolerance and overall plant vigour. Together, these nutrients are vital for maintaining healthy, sustainable soil ecosystems.

The findings indicate that the treatments involving combinations of Orgafol, NAA and microbial inoculants such as *Azospirillum*, AMF and phosphobacteria significantly enhanced the levels of available nitrogen, phosphorus and potassium in the soil. Specifically, treatments T9, T6 and T4 demonstrated the highest nutrient levels when applied at a rate of 20 g of pellet per plant. This suggests that microbial inoculants in pellet form have a profound impact on improving the nutrient status of the rhizosphere. The increase in NPK availability observed in these treatments is consistent with findings from other studies. For

Table 7. Eigenvalues, percentage variance and cumulative variability of soil properties

Soil properties	Principal components	Eigenvalues	Variance (%)	Cumulative (%)
EC	PC1	3.230	40.370	40.370
pH	PC2	2.632	32.895	73.265
N	PC3	0.980	12.248	85.513
P	PC4	0.545	6.816	92.329
K	PC5	0.397	4.965	97.294
Bacterial count	PC6	0.174	2.174	99.467
Fungal count	PC7	0.026	0.323	99.791
Actinomycetes count	PC8	0.017	0.209	100.000

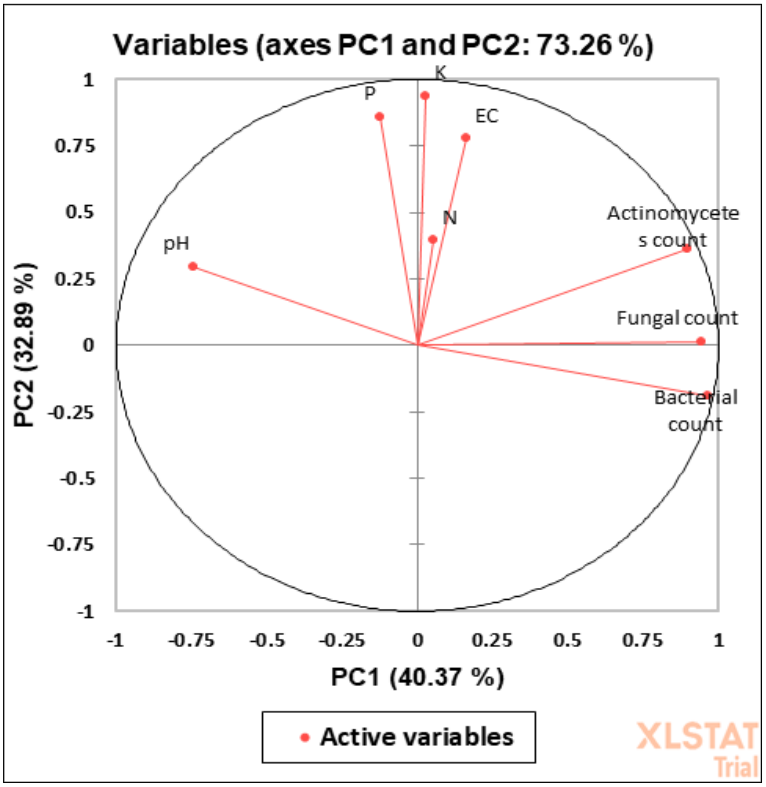

Fig. 7. Eigenvectors of soil chemical and microbial variables.

Table 8. Treatments selected based on principal components score greater than 1.0 value

	PC1	PC2
Treatments	T9P5 (2.967) T9P4 (2.890) T9P3 (2.854) T10P5 (2.820) T9P2 (2.662) T10P4 (2.616) T10P2 (2.592) T9P1 (2.528) T10P3 (2.522) T10P1 (2.491)	T6P5 (1.851) T6P4 (1.758) T6P3 (1.691) T1P5 (1.590) T6P2 (1.589) T1P4 (1.487) T6P1 (1.462) T1P3 (1.361) T9P5 (1.285) T1P2 (1.256) T4P5 (1.207) T9P4 (1.172) T1P1 (1.114) T4P4 (1.108) T9P3 (1.053) T10P5 (1.001) T4P3 (1.001)

instance, it has been discovered that increased NPK availability in soil could be due to the nitrogen-fixing ability of *Azospirillum brasilense* and phosphorus solubilization and mobilization by *A. awamori* and the presence of farmyard manure (FYM), vermicompost, green manure, oilcakes and inorganic fertilizers (5). Moreover, others reported that the nitrogen-fixing ability of *Azospirillum* improves soil fertility by increasing nitrogen levels (25). Apart from this, bio-inoculants capable of nitrogen fixation could increase soil nitrogen levels by a significant range (20 to 400 kg ha^{-1}) (26). Similar outcomes were reported suggesting an enhanced NPK availability when biofertilizers were used in conjunction with organic materials (27, 28).

Microbial inoculants, such as free-living cyanobacteria, *Azotobacter*, *Azospirillum* and *Pseudomonas* spp., have been documented to play vital roles in the global nitrogen cycle (29, 30). PSB not only improve phosphorus uptake and crop yield but also have multifaceted roles in promoting plant growth. According to some researcher, PSB enhance N, P and K nutrition, act as biocontrol agents against phytopathogenic fungi, synthesize phytohormones in the rhizosphere and ultimately stimulate plant growth and development (31). The ability of PSB to make phosphorus available from both organic and mineral sources further underscores their importance in sustainable agricultural practices. Overall, these findings highlight the significant role that microbial inoculant, especially when combined with organic amendments, play in enhancing soil nutrient status and, subsequently, plant growth. This approach represents a sustainable method of improving soil fertility by leveraging natural processes of nitrogen fixation, phosphorus solubilization and microbial activity to optimize nutrient availability in the soil.

Microbial populations in soil, including bacteria, fungi and actinomycetes, are crucial for soil health as they play key roles in decomposing organic matter, cycling nutrients and suppressing diseases. These microorganisms improve soil structure, enhance nutrient availability and boost soil fertility by breaking down organic matter and converting nutrients into plant-usable forms. A diverse and active microbial community is vital for sustaining a resilient soil ecosystem that supports healthy and sustainable plant growth.

The study highlights that the T9 treatment, consisting of Orgafol, NAA, *Azospirillum*, AMF and phosphobacteria, had a significantly positive impact on the soil microbial community compared to other treatments. This finding is consistent with several other studies across different crops such as in tea, rice and black pepper, demonstrating that a combination of organic and microbial inputs can greatly influence soil microbial dynamics (32-34). These studies collectively suggest that such treatments have broad applicability across various crop systems, influencing microbial populations in diverse agricultural contexts.

The increased organic carbon content in soil due to

organic manure application could be a key factor contributing to the observed improvement in microbial community structure and activity. Organic carbon serves as a primary energy source for soil microorganisms, promoting their growth and metabolic activities. This observation is in line with results reporting that, organic amendments significantly increase soil organic carbon, which in turn enhances microbial biomass and diversity (4).

Furthermore, the findings align with earlier research showing that the interaction between AMF and *Azotobacter* in the rhizosphere significantly increased population of beneficial soil bacteria and actinomycetes compared to when either was applied alone (35). The present study also corroborates the observations where it has been reported that soils inoculated with a combination of biofertilizers, including *Azospirillum*, phosphobacteria and AMF, exhibited a higher population of bacteria, fungi and actinomycetes compared to untreated soils (1). This suggests that the introduction of beneficial microorganisms through biofertilizers can enhance the overall microbial community, contributing to improved soil health and fertility.

Principal component analysis (PCA) is a statistical tool widely used to analyse changes in soil chemical properties. It is a multivariate technique that models the covariance structure of data by identifying latent variables that represent linear combinations of interrelated variables (36). PCA has been employed in numerous agricultural studies examining the chemical properties of soil and nutrient status of eucalyptus following biosolid application, the influence of irrigation water quality on various soil chemical, physical and biological properties, the characteristics of 19 soil profiles rich in organic material from different regions in Brazil, the key differences in the physical and chemical properties of Ultisols along the Brazilian Atlantic coast, corn yield variations in relation to soil properties and the physical and chemical properties of soils irrigated with wastewater (37-42).

In the present study, PCA was applied to evaluate the impact of pelleted biofertilizer at varying concentrations on soil chemical properties and microbial populations. Eight principal components were derived from the correlation matrix. Principal components with the Eigenvalues greater than 1.0 were considered significant and should be retained (43). The PCA results indicated that treatments T9 and T10 were the most influential for PC1, while T6 were dominant in PC2. This ranking highlights the most effective treatments, offering insights for further evaluation. Implementation can be tailored based on specific criteria such as soil health, microbial activity or plant growth parameters.

Conclusion

The findings of this study highlight the significant advantages of slow-release biofertilizer pellets in enhancing soil fertility over traditional chemical fertilizers. Treatments combining Orgafol with microbial inoculants such as *Azospirillum*, AMF, phosphobacteria and NAA significantly increased the availability of essential nutrients, including nitrogen, phosphorus and potassium. These biofertilizer pellets not only improved nutrient availability but also positively affected soil pH and microbial populations, contributing to a healthier and more balanced soil ecosystem. PCA analysis further demonstrated the effectiveness of these treatments, with T9 and T10 ranking highest in PC1 and T6 dominating PC2, underscoring their strong impact on soil fertility enhancement. Compared to chemical fertilizers, which provide a quick but transient nutrient boost, slow-release bio fertilizer pellets offer a more sustainable and environmentally friendly approach by ensuring a gradual and consistent nutrient supply. They also improve soil structure, enhance microbial diversity and mitigate the risks of nutrient leaching and soil degradation. Incorporating slow-release biofertilizer pellets into sericulture and other agricultural practices could lead to sustained improvements in soil fertility, productivity and environmental sustainability. These findings suggest that slow release biofertilizers are a viable alternative to traditional chemical fertilizers, offering long-term benefits for soil health and crop productivity. Future research should focus on the long-term effects of these biofertilizers and evaluate their potential for broader adoption across diverse cropping systems to support sustainable agricultural practices.

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Authors' contributions

IN conceived and designed the study, conducted the experiments and analyzed the data. SR contributed to the design of the study, provided essential materials and assisted with data interpretation. PRN performed the statistical analysis and contributed to data interpretation. PLD and GN assisted with laboratory work, data collection and manuscript preparation. MS and MHA contributed to drafting and revising the manuscript, provided critical feedback and approved the final version of the manuscript for submission. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

QuillBot and Grammarly were used to check grammar, improve sentence clarity and reduce unintentional plagiarism. These tools were employed to polish the language, rephrase sentences for better readability and ensure linguistic quality without altering the scientific meaning or originality of the content. After using this tool/service, the author reviewed and edited the content as needed and take full responsibility for the content of the publication.

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