International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 18(3): 348-360, 2025
doi:10.5281/zenodo.18067478
Research Article

EFFECTS OF SOLID AND LIQUID BIO-FERTILIZER ON *GLYCINE MAX* L. (SOYBEAN) CULTIVATION AND SOIL MICROBIOMES

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(Received 9th October 2025; accepted 12th December 2025)

ABSTRACT. Farmers are widely using biofertilizers due to public concern about the security and safety of food. The process of bio-fertilization alters the microbial composition of the rhizosphere by having microorganisms present in the soil with organic residues. It is assumed that one gram of biofertilizer-rich soil may contain up to 1010 cfu of bacteria. These plant-growth-promoting microbes developed a symbiotic association with the growing plants to supply a nutrient-rich soil reservoir. Consequently, the number of bacteria in the soil is affected by increasing the percentage of plant germination, the type of soil, and the amount of organic matter present. By considering these facts, the experiment was conducted to find out the effect of different biofertilizers on soil microbiomes and soybean production. Since soybean is a significant source of nutritious food and oil, more research is required to boost their output. Therefore, to achieve a significant production of soybeans globally, one must assess all the parameters responsible for the enhanced production, including their association with soil bacteria. In order to evaluate the impact of various treatments on soil microbiomes and soybean production, the study included five inoculum treatments to the soil: (1) chemical fertilizer, (2) cow dung fertilizer, (3) vermicompost, (4) liquid biofertilizer, (5) mixture of all biofertilizers, and one control treatment (C) without any nutrient supplement. Our findings indicated that adding cow dung to the soil resulted in the highest overall CFU count, followed by the addition of mixed biofertilizers. However, the vermicompost performed better in all aspects of growing parameters, such as nodulation and vegetative growth of the soybean. The control treatment proved to be the lowest performer in all parameters studied.

Keywords: *Biofertilizer, sustainability, insecticides, rhizosphere, vermicompost*

INTRODUCTION

Global food demand has increased as a result of population expansion and rising food consumption, while agriculturally productive land is getting harder to expand. Due to urbanization, the total amount of land used for food production has gradually decreased over the past few years. All of this suggests that the production of food will need to be increased far more intensively and effectively than it has been previously [1]. Agricultural land per unit area is necessary to attain optimum efficiency and the greatest possible food product quality in order to meet the world's expanding food demand. It is well known that plant nutrition is one of the most important factors in controlling agricultural productivity and quality. The amount of nutrients in the soil has an impact on the plant yield [2]. In order to address the challenges of food scarcity and to enhance soil nutrients, various agricultural alternatives, including the use of chemical fertilizers, insecticides, and pesticides, have been used to produce disease-free, high-yielding crops [3]. However, these synthetic fertilizers and insecticides raised public concern over the sustainability, security, and safety of the food. It is revealed that even when crops are removed from the farms, there is still a large concentration of pesticides and chemical fertilizer residues retained in the soil, uprooted plants, plant parts, and their products. Therefore, it is very important to use some alternatives to guarantee the security and safety of the food and soil [4]. The synthetic fertilizers that contain different nutrients like nitrogen (N_2), phosphorus (P), potassium (K), and sulphur (S), if utilized in excess, may become toxic not only to the plants but also to the soil [5]. Some chemical fertilisers have high radionuclide concentrations as well as heavy metals like cadmium and chromium. These chemical fertilisers are the primary source of heavy metals and radionuclides in plants. Stewart and Roberts [6] reported that some of these chemical fertilizers also cause an accumulation of inorganic contaminants in the soil and plants. The impact of these chemical fertilizers causes weakened plant roots and a high incidence of diseases, etc. These poisonous substances also cause soil acidification and eutrophication of groundwater and other water bodies. The post-effects of these chemicals will not only be felt by the current generation but also by generations to come [7]. The negative impact of chemical fertilizers begins with their manufacturing, where by-products in the form of certain harmful compounds and gases, such as NH4, CO2, and CH4, pollute the environment. Due to the extensive use of nitrogen fertilizer, nitrate levels in rivers and drinking water may rise. Similarly, the conveyance of phosphorous fertilizer with the flow of the surface, the concentration of phosphate in drinking water, and rivers may rise. According to Sönmez et al. [8], the nitrogen fertilizer contains nitrosamines, which are carcinogens that have accumulated in plants like spinach and lettuce leaves.

In some regions of the world, the usage of biofertilizers or microbial inoculants has significantly increased over the past 20 years [9]. In order to raise crop output, soil improvement and restore its fertility, promote plant development, minimize production cost, and reduce environmental pollution associated with chemical fertilizers, biofertilizers are seen as a viable and attractive biotechnology option [10, 11]. Numerous microorganisms, such as phosphate-solubilizing bacteria like *Pseudomonas*, nitrogen-fixing cyanobacteria like *Anabaena*, nitrogen-fixing soil bacteria like *Azotobacter* and *Rhizobium*, and vesicular arbuscular mycorrhiza (VAM) fungi, are frequently utilized as biofertilizers. The soil microorganisms can help develop strategies for encouraging plant growth under both normal and abiotic stress conditions [12, 13]. The addition of biofertilizer also alters the soil pH, which is one of the factors that influence phosphate (P) fixation. The biofertilizers increased the availability of Fe, Al, and Ca in soil, which helps in P fixation. In alkaline soil, the Ca^{2+} combines with phosphate ions to form different insoluble phosphate compounds, leading to the reduction of P availability. On the other hand, in highly acidic soil, Fe and Al react with phosphate ions to form hydroxyl phosphate precipitates, which eventually prohibit plants from accessing phosphate [14, 15]. However, the microorganisms solubilize the fixed phosphate by releasing organic acids, which ultimately reduces pH, through chelation activities. This facilitates the P adsorption at that site by releasing P through the formation of soluble complexes with metal ions. The low pH soil results in the most soluble form of monovalent phosphate, whereas high pH soil results in the conversion of monovalent to divalent and trivalent forms, which are highly insoluble. According to studies, phosphate-solubilizing bacteria produce gluconic acid, oxalic acid, tartaric acid, and lactic acid, which lower soil pH and generate ideal conditions for the formation of available monovalent forms [9]. The soil pH, organic matter, physical and chemical characteristics of soil, vegetation type, environmental conditions, agriculture practices, and interactions between microbes and crops all influence crop yield [16]. Considering all these points, one can conclude that biofertilizers support biodiversity in the soil, maintain ecological balance, and reduce the negative environmental impacts associated with intensive chemical-based farming practices. The effectiveness of biofertilizers may vary depending on factors such as crop type, soil conditions, and specific product formulations [17]. Therefore, it is recommended to choose and apply biofertilizers based on the specific requirements of the crops and the local agricultural conditions. Several crops like soybean (*Glycine max* L.) are generally associated with soil microbes and develop a symbiotic association for the exchange of various nutrients. The main aim of adapting these strategies is to enhance the livelihoods of the farmers. Due to the multiple uses of soybeans in terms of health benefits, the developing countries have supported their large-scale production. Bruinsma [18] stated that the production of soybeans has received support from developing countries as one of the numerous and diverse adaptation solutions aimed at improving the standards of living due to its multiple effects. The countries that are involved in the soybean production are the USA, Brazil, Argentina, China, and India [19, 20].

In recent years, in order to enhance the soybean yield, farmers enriched the soil with biofertilizer. Microbes present in biofertilizer may have important functions in soybean production as well as public health. Biofertilizers offer numerous benefits for soybean production or any other agricultural crop production, such as nutrient enrichment in soil and its health improvement, disease suppression and pest control, eco-friendly, and cost-effective. Biofertilizers are a sustainable alternative to chemical fertilizers. Overall, because they improve crop output, lessen their negative effects on the environment, and support long-term food security, biofertilizers are crucial to sustainable agriculture. Their use aligns with the principles of organic farming and supports the goal of achieving a more sustainable and resilient agricultural system [21, 22, 23]. Several methods have been developed to assure the greatest possible survival of the microorganisms used in bio-formulations, and a variety of commercial biofertilizers are now readily accessible [24]. These methods include the use of solid/liquid biofertilizers, vermicompost, manure, cow dung, etc., which consist of strains that are drought or heat-resistant or genetically modified. This paper explores the information relevant to the enhancement of soybean production by using various biofertilizers available in the market. It has been found that among the major factors affecting the soybean yield, the most important factor is the appropriate use of inocula or biofertilizer. The ecology of the soybean for management purposes requires a better understanding of the broad diversity in abiotic and biotic conditions. The experiment was conducted at St. Xavier's College, Ahmedabad, India to evaluate the effects of solid and liquid bio-fertilizers for soybean production.

MATERIALS AND METHODS

Treatments of soil

The soil samples were collected at random locations from the garden area of St. Xavier's College, Ahmedabad, Gujarat, India. For each test, a total of three replicates were taken. The impact of soil treatments was evaluated on the response of soybean seeds. The study comprised five inoculum treatments to the soil, viz., (1) chemical fertilizer (CF), (2) cow dung (CD), (3) vermicompost (V), (4) liquid biofertilizer (L), (5) mixture of all biofertilizers (Mix), and control (C) without any treatment. All five inocula were mixed in the soil at a ratio of 1:5 (5 g inoculum in 25 g soil). Each of these treated and untreated soil samples was taken in a plastic pot. An equal number of soybean seeds were sown in all the pots of each treatment and control. Precautions were taken while selecting the soybean seeds, so that all the seeds were fresh, healthy, and not broken or infected. To avoid contamination of the treatments, a separate plastic shovel and hand gloves were used for handling each treatment. The soil analysis and its microbial observation were done on the 0th day, 15th day, and 30th day from the day of seed sowing in each treatment.

Estimation of soil organic carbon (Mebius Method)

Estimation of soil organic carbon was done by using the Mebius method [25]. 1 g of soil sample was mixed with 10 ml of distilled water in a clean and dry test tube. Mixed them vigorously. Filtered the soil suspension through Whatman-42 filter paper. The soil filtrate was treated with 1N $K_2Cr_2O_7$ solution, concentrated H_2SO_4, water, and titrated against 0.2N ferrous ammonium sulfate using 2 to 3 drops of diphenylamine (DPA) as an indicator**.** The H_2SO_4 : soil suspension ratio and the milli-equivalents of dichromate used in the digestion of soil were used as per the Mebius protocol. The change of suspension filtrate from violet to green was the endpoint of the titration. The calculation of the organic content was done by using the equation:

$$\text{Organic Carbon (\%)} = \frac{(V1-V2) \times N \times 0.003}{W}$$

Where, V1 = Volume of Blank; V2 = Volume of soil filtrate; N = Normality of potassium dichromate (1N); 0.003 = Molecular weight of carbon; and W = Weight of soil sample

Estimation of available soil phosphorus (Olsen Method)

Estimation of phosphorus in soil was done by using Olsen method [26, 27]. 5 g of soil along with one teaspoon of activated charcoal and 100 ml 0.5M $NaHCO_3$ solution was mixed well in a 250 ml plastic bottle and incubated for 30 minutes on an orbital shaker. Suspension was immediately filtered through Whatman-42 filter paper. In a 25 ml volumetric flask, 5 ml aliquot, 5ml ammonium molybdate solution, and distilled water were taken and shaken well. The 1ml of $SnCl_2$ was added into it and finally the volume was made to 25 ml with distilled water. The optical density (OD) of the solution was measured at 660 nm wavelength in a UV spectrophotometer (Shimadzu) at two different time periods, first, 20 min. before and second, 5 min. after the addition of $SnCl_2$ solution.

Estimation of soil nitrogen (Kjeldahl Method)

Estimation of nitrogen in soil was done by using kjeldahl method [28]. 1 g of the air dried soil sample was taken in a kjeldahl flask. In the presence of sulfuric acid, the soil sample was heated. For the purpose of raising the boiling point of the content, a pinch of potassium sulphate was added. Once the content had completely digested, a clear, colour-less solution was produced. A pinch of sodium hydroxide was added to the digested solution to turn the ammonium salt into ammonia. The vapours from the distillation process are then mixed with water and HCl (hydrochloric acid) solution. The amount of nitrogen or ammonia in the sample is then calculated using back titration. Some HCl was neutralized as the ammonia dissolved in the acid-trapping solution. The remaining acid was titrated with a NaOH solution. The titration values were recorded.

Estimation of soil pH

1g of soil sample was mixed with 10 ml of distilled water in a clean, dry test tube. Mixed them vigorously. A pH strip was dipped in the soil suspension. Colour was observed on the pH strip to find out the pH of the soil suspension. The pH value was recorded.

Isolation of total soil microflora

1g of soil was suspended in 10 ml of sterile distilled water to make soil suspension. 1 ml of soil suspension was used to make serial dilutions of 10^{-1} to 10^{-6}. The 10^{-6} soil suspension dilution was used to isolate soil bacteria. 0.1 ml of soil suspension was spread on the sterile nutrient agar medium plate with the help of a sterile spreader. All petri plates were incubated at room temperature for 24 hours. The bacterial colonies were observed on the nutrient agar medium.

Isolation of phosphate solubilizing bacteria

0.1 ml of microbial suspension was spread on the sterile Pikovskaya (PVK) medium plates with the help of a sterile spreader. All petri plates were incubated at room temperature for 24 to 48 hours.

Isolation of nitrogen-fixing bacteria

0.1 ml of microbial suspension was spread on the sterile Ashby's mannitol agar medium plates with the help of a sterile spreader. All petri plates were incubated at room temperature for 24 hours.

RESULTS AND DISCUSSION

Estimation of organic carbon

The primary factor that affects soil quality, physical stability, and fertility is soil organic carbon (SOC). The SOC changes the physico-chemical and biological characteristics of the soil, improving soil aeration, water drainage, and retention, and reducing the risk of nutrient leaching. In Fig. 1, our data shows the maximum percentage of organic carbon observed in cow dung and mixed biofertilizer (7.0%), and the least in control (4.5%) and chemical fertilizers (4.0%) on the 30th day of treatment. Liquid fertilizer and vermicompost also showed a marginal increase in the percentage of organic carbon after 30 days of application.

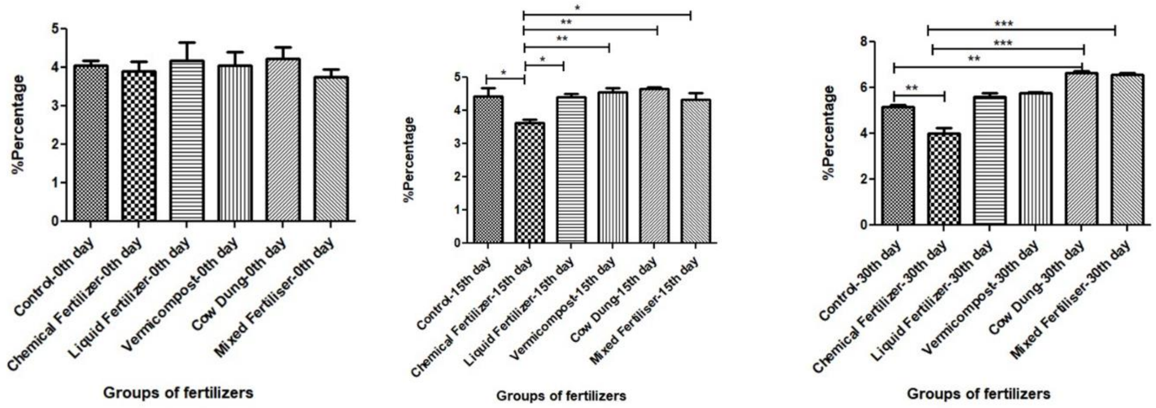

Fig. 1. *Estimation of carbon in treated and control soil suspension on the 0th, 15th, and 30th day of treatment. Mean values ±SE *, **, *** indicate significant difference at P≤ 0.05, P ≤0.01, and P ≤0.001 respectively. n =3.*

Peinemann and his co-workers [29] reported that soils containing greater than 12–18% organic carbon are generally classified as organic soils. In general, the climate, temperature, and humidity highly influence soil carbon content.

Estimation of total phosphorus

When comparing mixed biofertilizer to the chemical fertilizer, it has been shown that the highest amount of available phosphorus is present in mixed biofertilizer (9 kg/ha) after 30 days of treatment shown in Fig. 2. Furthermore, it has significantly increased in comparison to chemical fertilizer. The control was shown to have almost 13 kg/ha of available phosphorus. Researchers reported that phosphate adsorption by soils increases with reaction time, first rapidly and then slowly, but without reaching a true equilibrium [30]. Soil organic matter, iron oxide and aluminum oxide of soil are also responsible to decrease the available phosphorus. The availability of phosphate in the soil is substantially influenced by precipitation and dissolution processes. Phosphate minerals can dissolve and replace the phosphate in the soil solution over time.

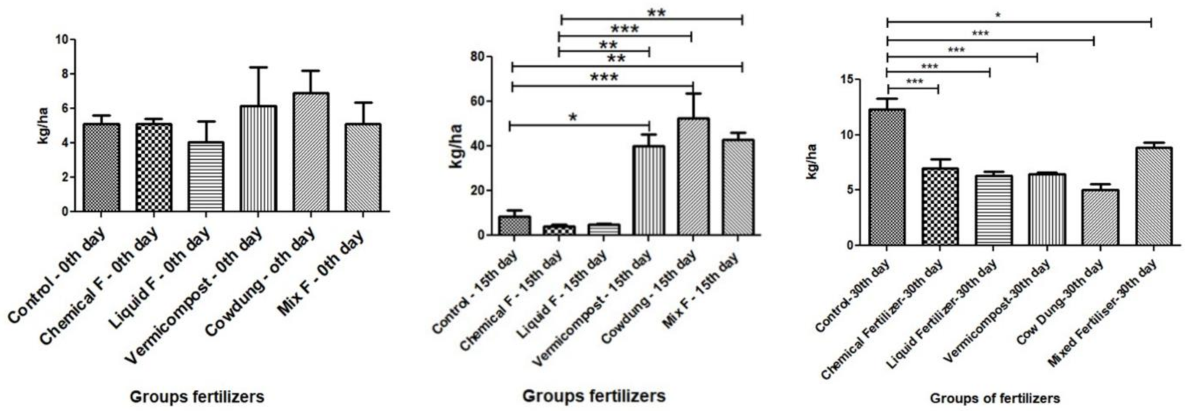

Fig. 2. *Estimation of phosphorus in treated and control soil suspension on the 0th, 15th, and 30th day of treatment. Mean values ±SE *, **, *** indicate significant difference at P≤ 0.05, P ≤0.01 and P ≤0.001 respectively. n =3.*

This process enhances phosphorus availability. Phosphate minerals are formed by extracting phosphate from the soil solution. This process reduces phosphorus availability. Precipitation and dissolution are extremely slow processes. Besides, a large volume of phosphate is used in the soybean seeds as a major nutrient for its growth and development process. Therefore, the phosphorus was so high on the 15th day and drastically reduced on the 30th day.

Estimation of total nitrogen

Our data shows in Fig. 3 that the amount of total nitrogen in chemical fertilizer, liquid fertilizers and mixed fertilizers is increasing (approximately 350 kg/ha) as compared to cow dung, vermicompost and control (250-300 kg/ha) on the 30th day of treatment. There was a low level of significance observed. Microorganisms use available nitrogen from nitrate in the soil to proliferate seed germination. Microorganisms present in the organic fertilizers such as liquid, vermicompost, cow dung, and mixed fertilizer explain the lower nitrogen levels in soil recovered from these populations.

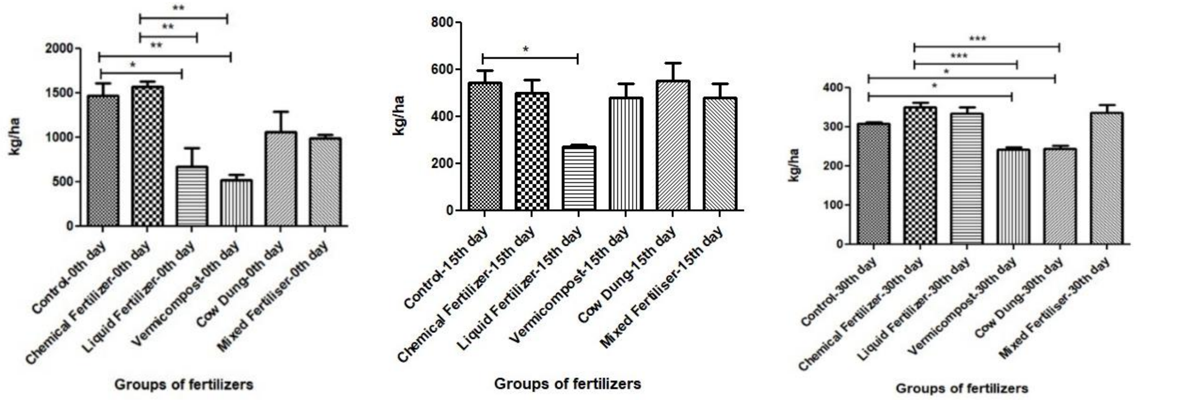

Fig. 3. *Estimation of total nitrogen in treated and control soil suspension on 0th, 15th, and 30th day of treatment. Mean values ±SE *, **, *** indicate significant difference at P≤ 0.05, P ≤0.01 and P ≤0.001 respectively. n =3.*

Even at the same ratios of the fertilizer and soil (1:5) in all the treatments, the nitrogen concentration on the 0th day showed variation in the nitrogen concentration. This is due to the difference in the concentration of total nitrogen in each of the inoculum fertilizers. The total number of soybean seeds placed in the treated soil samples was the same; the nitrate used in the soybean seeds was a major nutrient for its growth and development process. Therefore, the total nitrogen was high on the 15th day and drastically reduced on the 30th day.

Estimation of soil pH

The 0th day of soil treatment with cow dung showed an acidic pH, while the other groups had almost neutral pH, as shown in Fig. 4. On the 15th day, the liquid fertilizer and vermicompost had a pH of 7.0, whereas none of the groups showed an acidic pH. On the 30th day, the chemical fertilizer showed an acidic pH of 5.5, while the other group of fertilizers had a neutral pH.

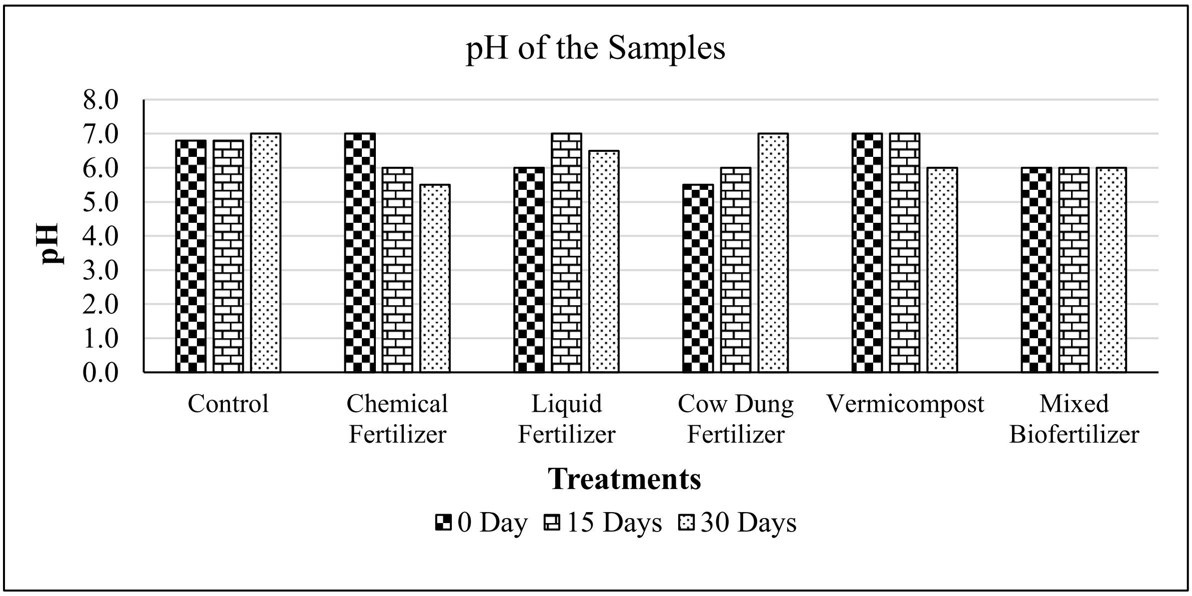

Fig. 4. *Estimation of soil pH in treated and control soil suspension on 0th, 15th, and 30th day of treatment.*

The conversion of ammonium-containing compounds to nitrate causes acidity in the soil. As more nitrogen fertilizer based on ammonia is applied, the soil becomes more acidic. It is reported that the amount of annual nitrogen fertilization is typically correlated with soil pH and organic carbon [31]. The authors observed that soil pH was dramatically lowered by long-term nitrogen fertilization. In general, maintaining the pH at 5.5 to 7.5 is recommended for a good level of fertile soil. If pH of soil goes less than 5.5, then it is not good for the plants and make them difficult to absorb nutrients from the soil. The initial decrease in pH was attributable to the generation of a few organic acids in earthworm-mediated vermicompost [32], but after 30 days, there was a decrease in pH observed in almost all the treatments. This was essentially due to the high level of phosphorus stabilization.

Isolation of total soil microflora

The 15 day's treatment revealed that a total of 325 microbial colonies were formed in the cow dung biofertilizer treated soil samples which is the highest among all treatments shown in Fig. 5. Hence, cow dung biofertilizer significantly increased the soil microflora. Next to the cow dung, there was mixed biofertilizer (290 cfu) followed by liquid biofertilizer (145 cfu) and vermicompost (104 cfu) shown to have the good number of cfu count. There is a marginal difference of cfu count observed between vermicompost and chemical biofertilizers. Control is shown to have the least number of microbial colonies formation.

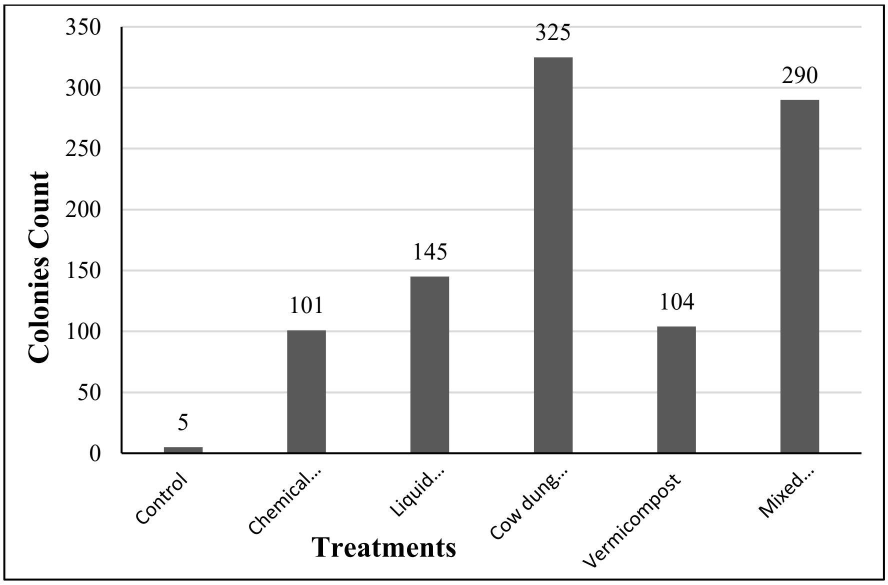

Fig. 5. *Total number of microbial colonies observed after 15 days of fertilizer-treated and control soil samples.*

The excessive and unilateral use of synthetic fertilizers has not only negatively impacted the environment but also the beneficial microflora of the soil. The activity of these microflora in the soil is crucial for the conversion of organic matter into nutrients for the better growth and development of the plants and to improve the yield [33]. The unreasonable use of chemical fertilizers caused food security issues and reduced the population of soil microflora, enhancing plant performance. However, organic fertilizers, such as agricultural fertilizers, enrich soil nutrients and boost plant yield and microflora populations. Crop growth, production, and quality can all be enhanced by using biologically treated soil [34].

Isolation of phosphate solubilizing bacteria

Phosphate-solubilizing bacteria were isolated on Pikovskaya agar medium. Pikovskaya agar is a selective and differential medium commonly used for the isolation and enumeration of phosphate-solubilizing bacteria (PSB) from soil or any other environmental sample [35]. It contains specific components that facilitate the growth and detection of these bacteria based on their ability to solubilize inorganic phosphate. The Pikovskaya agar has the ability to detect the production of an enzyme called phosphatase by PSB. Phosphatase enzymes are secreted by these bacteria to break down organic phosphorus compounds and convert them into soluble inorganic phosphate, which can be easily absorbed by plants. The solubilization of inorganic phosphate is an essential trait of PSB that contributes to plant growth and nutrient availability [36]. The bulk of crucial plant nutrients, including phosphorus, are not soluble in soil. The phosphate-solubilizing bacteria added to the soil help the crops uptake applied phosphates and fixed soil phosphorus, leading to increased crop yields [37]. The synthesis of organic acids has been linked by several authors to a particular set of bacteria that are involved in the solubilization of inorganic insoluble phosphate [38]. The PSB isolated from the 10^{-4} dilution of soil suspension showed some specific morphological characteristics while grown on Pikovskaya agar medium, as listed in Table 1. Our data shows that in all the treatments from the 0th to the 15th day, there was a significant increase in the number of PSB colonies. However, from the 15th to 30th day, there was no subsequent increase in the number of colonies for PSB shown in Table 2.

Table 1. *Morphological characteristics of the phosphate solubilizing bacteria.*

Colour	Whole Colony	Margins	Texture	Elevation	Property	Size
Brown	Circular	Entire	Smooth	Raised	Opaque	Small
Light Brown	Circular	Entire	Smooth	Convex	Opaque	Small
Brown	Irregular	Lobate	Rough	Umbonate	Opaque	Large
Brown	Circular	Entire	Rough	Umbonate	Opaque	Large
Brown	Irregular	Lobate	Smooth	Flat	Transparent	Large
White	Circular	Entire	Smooth	Convex	Opaque	Small

Table 2. *Phosphate-solubilizing bacteria (PSB) colonies count on Pikovskaya agar medium.*

Groups	Colonies on 15th day	Colonies on 30th day
Control	1	2
Chemical fertilizer	8	8
Liquid fertilizer	56	45
Cow dung fertilizer	15	12
Vermicompost	19	17
Mixed fertilizer	13	11

Phosphate-solubilizing bacteria are slow growers. Environmental stresses that are common in degraded ecosystems like alkaline/saline soils, such as high temperatures, pH levels, salt, etc., have a significant negative impact on these bacteria. As a result, any change to the regular environmental conditions results in poor PSB growth and survival [39].

Isolation of nitrogen-fixing bacteria

To isolate nitrogen-fixing bacteria from soil, specific media and techniques are used. One commonly employed medium is the nitrogen-free or nitrogen-limited media. These media lack a readily available nitrogen source, forcing the bacteria to fix atmospheric nitrogen to meet their nitrogen requirements. The most commonly used nitrogen-free or nitrogen-limited media for isolating nitrogen-fixing bacteria are Jensen's medium, Ashby's medium, and Winogradsky's medium. We have used Ashby's medium for the isolation of nitrogen fixing bacteria from the treated and untreated soil samples. The nitrogen fixing bacteria were shown to have some specific morphological characteristics while grown on Ashby's agar medium listed in Table 3.

Table 3. *Morphological characteristics of the nitrogen fixing bacteria isolated on Ashby's mannitol media.*

Colour	Whole Colony	Margin	Texture	Elevation	Property	Size
White	Circular	Entire	Smooth	Raised	Transparent	Large
Yellow	Circular	Entire	Smooth	Raised	Opaque	Small
Pink	Circular	Entire	Smooth	Raised	Transparent	Small
White	Circular	Entire	Smooth	Raised	Opaque	Small
White	Irregular	Lobate	Smooth	Flat	Transparent	Large
White	Irregular	Lobate	Smooth	Flat	Opaque	Small
White	Circular	Entire	Rough	Umbonate	Transparent	Large
White	Irregular	Lobate	Smooth	Raised	Transparent	Large
White	Rhizoid	Undulate	Rough	Unbonate	Opaque	Large
White	Circular	Entire	Dry Powder	Unbonate	Opaque	Large
Orange	Circular	Entire	Smooth	Convex	Transparent	Small

In all the treatments, there was a subsequent increase in the nitrogen-fixing bacteria from the 15th to the 30th day of treatment, as shown in Table 4. Maximum bacterial colonies were observed in liquid fertilizer and mixed biofertilizer compared to control and chemical fertilizer.

Table 4. *Nitrogen fixing bacteria (NFB) colonies count on Ashby's mannitol medium.*

Groups	Colonies on 15th day	Colonies on 30th day
Control	380	550
Chemical fertilizer	460	980
Liquid fertilizer	511	1201
Cow dung fertilizer	411	1092
Vermicompost	175	1090
Mixed fertilizer	979	1101

Free-living microbes that fix nitrogen have frequently been described as promoting plant growth. By sharing a niche and giving bacteria fixed carbon in return for fixed nitrogen, plants and bacteria can profit equally from a mutualistic connection known as symbiotic nitrogen fixation [40]. The microorganisms take the available nitrogen coming from nitrate in the soil for their growth, which is observed through the increased level of germination in organic fertilizers like liquid, vermicompost, cow dung, and mixed fertilizer. This may explain the decreased level of nitrogen in soil extracted from these groups.

Soybean response in soil samples

All the soil samples were added with fertilizers at the ratio of 1:5 (5 g fertilizer in 25 g soil). 100 seeds were sown in all the soil samples (treated and control). Table 5 shows the data on the response of soybean seeds and plantlets found in each soil sample. In general, the presence of chemical fertilizers inhibits germination. When the chemical fertilizer is blended with the soil and comes into direct touch with the seed, this inhibition is stronger. Therefore, there was no germination of seeds that occurred with chemical fertilizer and mixed biofertilizer. The highest seed germination, shoot length, root length, and maximum number of leaves were observed in vermicompost-treated soil. Vermicompost organic fertilizer promotes plant growth regardless of nutrient availability and transformations. Vermicompost, whether added to soil or used as part of horticulture soilless container medium, has continuously increased plant productivity, seed germination, and seedling growth and development [41, 42].

Table 5. *Soybean response soil samples*

Sr. No.	Treatments	Seeds germination (%)	Shoot Length (cm)	Root length (cm)	No. of leaves
1	Control	34	5.5	2	2
2	Chemical fertilizer	0	0	0	0
3	Liquid biofertilizer	35	3	2.5	1
4	Cow dung	52	4	2.9	3
5	Vermicompost	60	6	3.5	4
6	Mixed Biofertilizer	0	0	0	0

CONCLUSION

Organic biofertilizers are available in many forms, such as liquid, solid, semi-solid, or mixed. Biofertilizers help increase soil microflora and promote soil fertility. In recent years, chemical fertilizers were widely used in agricultural practices and led to a decrease in soil microflora, thereby reducing soil fertility. Our data indicated that there is a higher level of phosphorus and organic carbon present in the vermicompost, cow dung, and mixed biofertilizer-treated soil as compared to the control and chemical fertilizer treatments. As a result, the vermicompost- and cow dung-treated soils showed the best growth response in soybean seeds after 30 days of application. These biofertilizers increase overall macronutrients and organic carbon in the soil. The maximum shoot length, root length, and germination percentage were observed in vermicompost. In comparison to chemical fertilizers, vermicompost has a high ability to produce a good amount of essential nutrients that are useful for the growth, yield, and quality of soybean. In general, organic biofertilizers have the ability to increase and enhance growth-promoting microbes as well as produce essential nutrients that favor the growth, development, and yield of almost all plants. Thus, organic biofertilizers can be used as a sustainable alternative to chemical fertilizers because they are excellent growth promoters and protectors for crop plants, prevent soil degradation caused by chemical fertilizer overuse, and are environmentally friendly. The use of organic biofertilizers has led to several benefits, such as increased levels of soil microflora, nitrogen fixers, and micronutrients such as phosphorus and carbon. They also maintain a neutral soil pH and increase seed germination, along with shoot and root length in soybean plants. The greater use of biofertilizers with plant growth-promoting microbes is a new field of study to deliver a sustainable environment and the development of eco-friendly farming for better and healthier living.

Acknowledgments. This work was performed under the auspices of the St. Xavier's College, Navrangpura, Ahmedabad, Gujarat, India.

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