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# The Effects of *Rhizobium phaseoli* and Phosphate-Solubilizing Microorganisms on *Phaseolus vulgaris* Amended with Rock Phosphate and Biogas Spent Slurry

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**Abstract**

*Phaseolusvulgaries* seeds were inoculated with *Rhizobium phaseoli*, Phosphate solubilizing Microorganisms (PSM) with rock phosphate in plots containing poultry droppings slurry of biogas plant. This experiment was carried out in 4 treatments with control (T1, T2, T3, T4 and T0).The samples were collected at seedling, preflowering, blooming and end stages to learn the phenotypical nature, NPK and chlorophyll contents. Studies on microbes have also been conducted on plants at every stage of development. Comparing the plant supplied by *R.phaseous* PSM with rock phosphate to control and other application combinations, the results showed that the plant recorded the highest values in the morphological, biochemical, and microbiological features.From the seedling stage on, the plants' NPK content also rose. (1.262%, 0.28% & 0.26%) to the flowering stage. In the T4 treatment, there are likewise 36 nodules, and each nodule weighs 0.268 g. The weight is roughly 0.039g, and the lowest nodule number (07nos) was discovered in the control. The enriched manure that produced the best results was the combination of SPDS+PSM+RP+*R.phaseoli* (T4). The yield was 2.435tons/hectare and PSM+RP (T2), which produced 1.890 tons/hectare; PSM+RP (T3), 1.101 tons/hectare in inorganic manure (T1), and control (0.821 tons/hectare) (T0). The study report highlighted the critical roles that PSO, *R. phaseoli,* and rock phosphate played in *P. vulgaries* nodulation, nitrogen fixation, and good yield.

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**Introduction**

India's agriculture industry has the ability to improve the nation's standard of living. The agriculture industry continues to be the main source of income for over 58% of the population in India, although contributing only 14.2% of the country's GDP, according to the country's most recent census (2011). But with time, the proportion of large farms (> 5 ha) fell from 6% to 3%, and the proportion of small farms (< 1 ha) rose from 59% to 67% between 1991 and 2011.[1] Because it barely occupies 2.3% of the world's land area, the nation faces an even greater difficulty in ensuring the food security of its fuming millions, or roughly 17.5% of the global population.[2] However, India uses 165 kg of fertilizer per hectare, compared to the global average of 138 kg. This indicates that fertilizer is utilized excessively and inefficiently, which increases the risk of insect infestation, soil pollution, and crop nutrition issues.[3,4]

**Chemical Agriculture's Repercussions**

More harm than good has resulted from chemically input based agriculture, despite claims to the contrary. Some of the well-known effects of chemical-based agriculture are global warming, soil micronutrient erosion, nitrification of ground water, pesticides getting into the food chain, etc. The role of naturally existing microorganisms has been hampered by previous trends in conventional Indian agriculture, such as monoculture without crop rotation, excessive use of chemical fertilizers, and widespread use of broad-spectrum organophosphate insecticides.[5] The beginning of the green revolution and the growing use of chemical fertilizers in agriculture were supposed to make the nation self-sufficient in food production, but instead they had a negative influence on the environment and all living things. The overuse of chemical fertilizers in agriculture is expensive and has a number of negative impacts on the soil, including a reduction in soil fertility, water-holding capacity, and nutrient disparities.[6]

The growing price of nitrogenous fertilizers, which is sometimes concealed by developing country governments as subsidies, further complicates the situation. A global manufacturer of nitrogenous fertilizers acknowledges this, stating that "natural gas is used as a raw material in the production of nitrogen fertilizers." Furthermore, a lot of heat is needed for the process, which can also be provided by natural gas. However, the truth remains that in order to feed the expanding populations of developing nations, more crops must be produced and the fertility of the land must be increased.[7] Therefore, it became necessary to create some inexpensive, environmentally acceptable fertilizers that would function without interfering with the natural world. These days, several species of microorganisms are employed extensively because of their special capacity to produce natural compounds that could be a useful alternative to chemical fertilizers. BgM is highly relevant as an input to sustainable agriculture (MNES)[8] because of all these factors.

**Biogas Manure (BgM) and its Features**

After dung or other biomass is digested to produce methane-rich gas, the biogas plant produces biogas manure (BgM), a byproduct. BgM provides vital nutrients, improves soil aeration and water retention, speeds up root development, and prevents weed seeds from sprouting.It might be feasible to apply biogas slurry and biofertilizer to fields in order to make use of the nutrients in the slurry and reduce the risk to the environment.[9] One important way to assess the quality of the ecological environment is to look at how bacterial populations respond to the usage of biogas slurry and biofertilizer.[10]

One of the key macronutrients required for healthy plant growth is phosphorus.[11] According to reports, phosphorus is the limiting plant nutritional component in soils and is essential for the appropriate feeding of plants. A significant amount of the accessible phosphate in soil is transformed into insoluble forms by microbes and chemicals, which gradually accumulates in the soil as an insoluble phosphate pool.[12] Rhizosphere soil often contains a significantly higher concentration of phosphate solubilizing bacteria. Phosphates are known to be soluble in a variety of microorganisms, such as *cyanobacteria*, *actinomycetes,* fungi, and yeasts.[13] The most potent phosphate solubilizers are *Pseudomonas, Bacillus, Actinomycetes, Cyanobacteria*, *Rhizobium, Penicillium* and *Aspergillus*. These microorganisms can release organic acids such as fumaric, fumaric, lactic, acetic, propionic, and formic acids, which can change the insoluble phosphates in soil into soluble forms. These acids dissolve bound phosphate forms and lower pH levels.[14]

Improving soil management, cropping techniques, and the inoculation of a highly effective *Rhizobium* strain are essential for increasing the seed production of leguminous crops, especially in Asia.[15] Nitrogen with phosphorus is two of the fertilizer ingredients that are essential to plant growth and development. One frequent soil bacterium is *Rhizobium.* Humans, plants, or animals cannot be poisoned by *Rhizobium*.[16] It is among the most advantageous microorganisms for farming. Either there aren't enough native *Rhizobium* plants to nodulate the crop appropriately, or the plants aren't doing a good job of fixing nitrogen. After years of producing legumes, *Rhizobium* will spread to neighbouring fields, and inoculation is cheap.[17] Throughout history, *Rhizobium* species have been incorporated into legumes as a means of providing fixed nitrogen.[18] Rhizobia, soil bacteria, work in symbiotic relationships with legumes to fix atmospheric nitrogen. Normally, they penetrate the root hairs, grow there, and produce nodules. The *Rhizobium* strain, the type of plant, and the surrounding circumstances all played a vital role how much nitrogen is fixed.[19]

**Top state for using Biofertilizers - Tamil Nadu**

In terms of using bio-fertilizers, which are organic fertilizers instead of chemical ones, Tamil Nadu leads all other states in this regard. The State produces more than 14,000 biofertilizers annually, according to data provided by the National Centre of Organic Farming.[20] Thus, an investigation was conducted to determine the significance of *Rhizobium*, PSO, and rock phosphate on the leguminous plant *Phaseolusvulgaries*, as well as the effects on the plant's microbial load, growth, and nutrition (Fig.1).

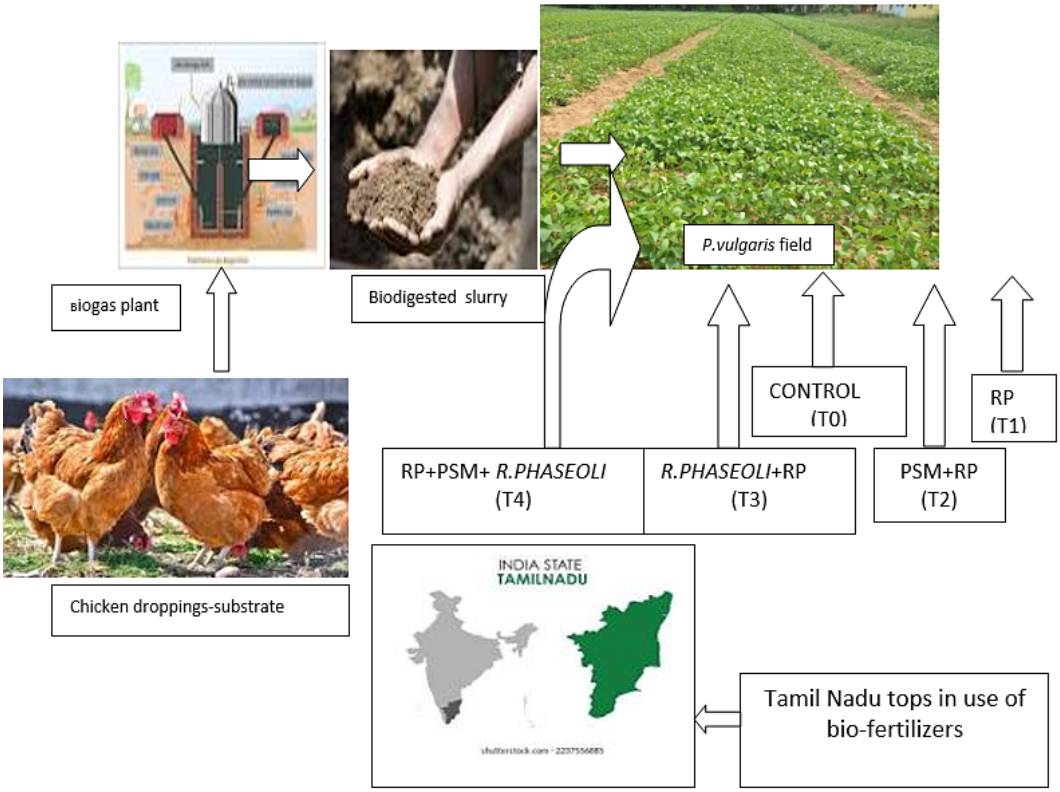



**Fig. 1: Graphical abstract of the study**

## Materials and Methods

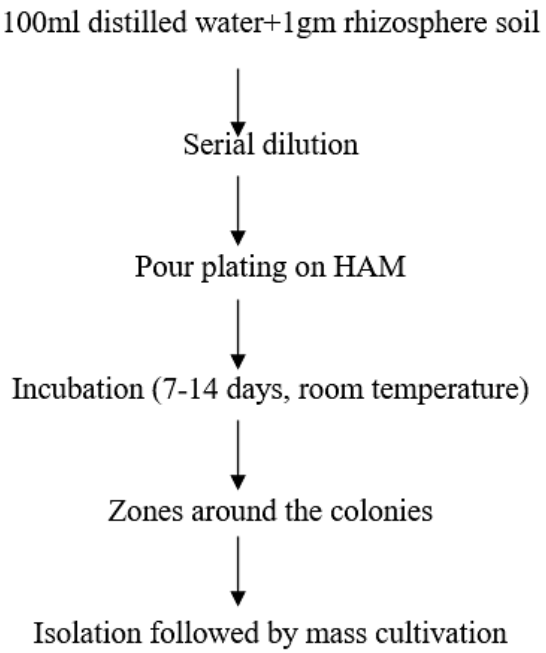
### Isolation of Phosphate Solubilizing Organisms

Isolation methodology was followed by Kannan, (1996)[21]

### Isolation of *R.phaseoli* from *P.vulgaris*

The leguminous plant was removed from Sankarankoil, Tamil Nadu, India, agricultural area, and the

soil particles that had adhered to the root system were washed away with flowing water. After that, the plant's shoot section was chopped off, and the nodule-filled roots were transported in polythene bags to the lab in order to isolate *R.phaseoli*.




A selection of firm, healthy pink nodules was made, and they were cleaned using tap water.

- For 4-5 minutes, they were submerged in 0.1% acidified HgCl2 to sterilize their surfaces.
- Using a glass rod and a tiny aliquot of sterile water, the nodules were crushed in a mortar. We refer to this as nodule extract.
- Up to $10^9$ serial dilutions were made. Decimally diluted nodule extracts are dispersed in aliquots over CRYEMA at the proper dilutions[22]
- For 3–4 days, the plates were incubated at room temperature (20–26$^0$C).

Large, gummy bacterial colonies appeared following a 5-day incubation period. They were verified, separated, cleaned, and kept in storage at 4ºC for future identification.

**Identification**

Vincent (1970)[22] provided a scheme for the cultural and biochemical testing of isolated rhizobial colonies. After being moved to nutrient broth, the pure cultures of R. phaseoli were agitatedly incubated for twenty-four hours at 37±2$^0$C. Several identifying techniques were applied to these newly created broth cultures. (Table 2).

**Sector Application**

Using soil as the substrate, the biogas waste slurry was used in the plot culture experiment. Rock phosphate was added to the plots containing biogas waste slurry at a rate of 10g P/kg of slurry. Before planting, water was added to each plot to change the slurry's moisture level. After rinsing in sterile water, the *P. vulgaris* seeds of uniform size were surface sterilized using a 0.5% sodium hypochlorite solution. In distinct plots, the seeds were planted and allowed to sprout. The plants were watered regularly to maintain 60 percent maximum Water Holding Capacity (WHC). On alternative days, the plots were inoculated with *R.phaseoli* and PSO for 10days. Germination count was taken for 10days after sowing. Plant growth parameters, nutrient content and microbial load were determined. The dry weight was recorded after drying in an oven at 600C for four days. Nutrient content in shoots were determined by the standard method of Jackson (1970).[23]

**Table 1: Different treatments for experimental plot plants**

| Treatment | Nature of the treatment |
|---|---|
| TO | CONTROL (SOIL&SLURRY) |
| T1 | ROCK PHOSPHATE (RP) |
| T2 | PHOSPHATE SOLUBILIZING ORGANISM (PSM)+RP |
| T3 | *R.PHASEOLI*+RP |
| T4 | RP+PSM+ R.PHASEOLI |

**Analytical Statistics**

The means (±SD) of three samples are used to express each value. Furthermore, a Least Significant Difference (LSD) analysis was performed on the preflowering, flowering, and final stage data. The findings showed that there was significant observation in the final stage and during flowering (Table 10). However, there was no significant observation in the pre-flowering stage because at

this stage the plants prepare themselves to accept the manurial effect, and their efficacy was only observed in the final stage and during flowering. Therefore, manure had no discernible influence during the preflowering stage but had a considerable effect throughout the flowering and final stages.

**Results**

**Table 2: Biochemical characteristics of R.phaseoli**

| Biochemical experiments | Observation |
|---|---|
| Microscopic observation | G(-) |
| Motility | Motile |
| Growth on Peptone-Glucose Agar | Very poor growth |
| Congo Red Test | white translucent, glistening colonies with entire margin |
| Hofer's Alkaline Broth Test | - |
| Lactose Agar | + |
| Catalase test | + |
| Starch hydrolysis | - |
| Casein hydrolysis | - |
| Lipid hydrolysis | - |
| Gelatin hydrolysis | - |
| Production of indole | - |
| MR-Test | - |
| VP-Test | + |
| Simmons citrate Test | + |

**TPSMP, THFP, and THBP analysis in the plot soil (CFU/ml)**

After collecting and analysing the rhizosphere soil from mud plots, it was discovered that THBP and THFP occurred in the range of $10^6$ and $10^3$ cfu/gm, respectively. The microbial density in the TPSMP example was approximately $10^3$cfu/gm. The field soil's THBP was 14x106±1.180cfu/gm, THFP was 16x$10^3$±1.431cfu/gm, and TPSMP was 41x103±1.078 cfu/gm prior to the amendments being applied. (Table 3).

**Table 3: THBP, THFP and TPSMP in the plot soil (CFU/ml).**

| Sampling stage | THBP | THFP | TPSMP |
|---|---|---|---|
| Initial soil | 14x$10^6$±1.180 | 16x$10^3$±1.431 | 41x$10^3$ ±1.078 |

THBP - Values as counts of x$10^6$cfu/gm
THFP&TPSMP - Values as counts of x$10^3$cfu/gm

**Examination of TPSMP, THFP, and THBP at different stages of treatment during the seedling stage (CFU/ml)**

The results revealed that the control consists lower microbial population (Fig. 2).The highest THBP, THFP and TPSMP were observed in the *R.phaseoli*+PSO+RP applied plots in the order (194x106±0.264cfu/gm),(Fig. 2A) (89x103±0.637cfu/gm) (Fig.2B) and (158x103±0.390cfu/gm) (Fig. 2C) respectively. The minimum load observed in control (29x103±0.415cfu/ml).

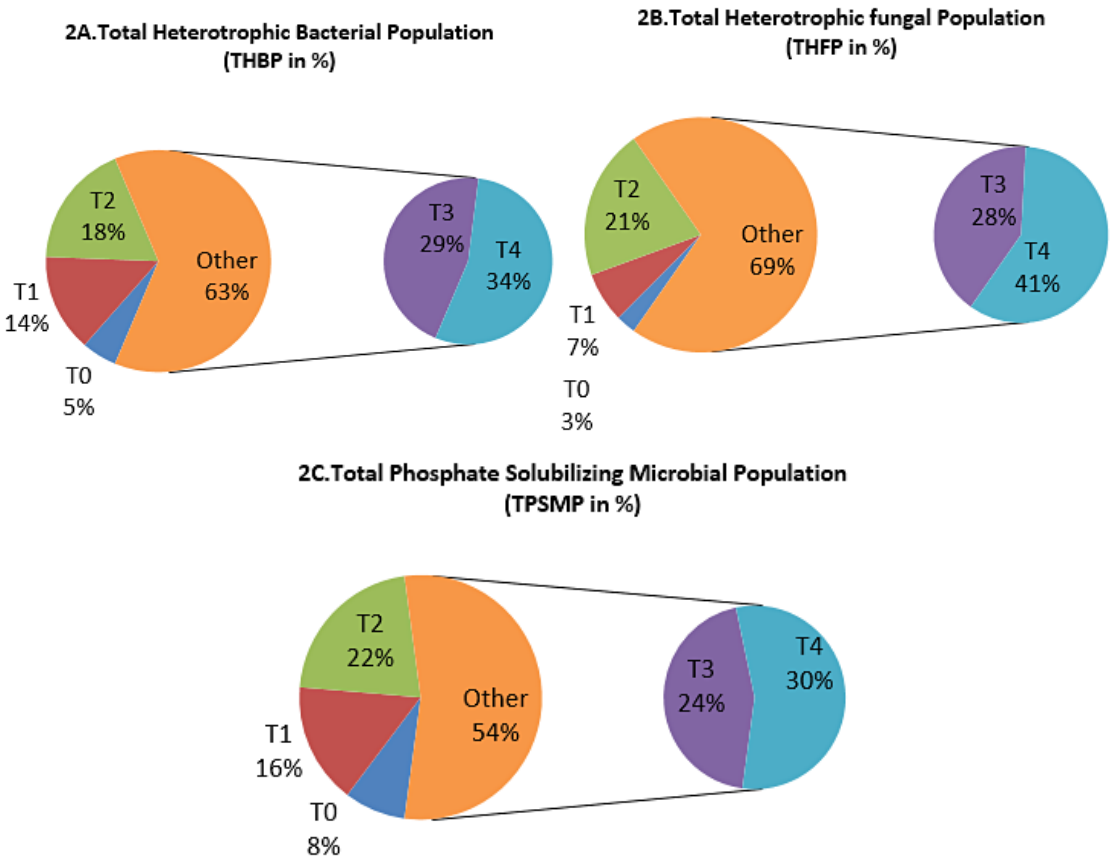
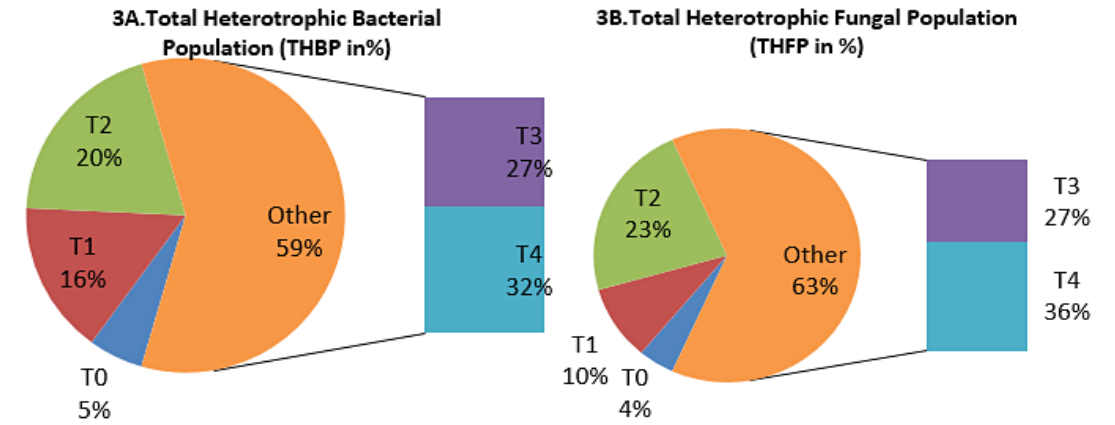



**Fig. 2: Analysis of THBP, THFP and TPSMP at various treatment during seedling stage(CFU/ml)**

**Pre-flowering stage analysis of THBP, THFP, and TPSMP at different treatments (CFU/ml)**

The analysis of microbial load of THBP, THFP and TPSMP during preflowering stage revealed that the maximum load was observed in the treatment plot of *R.phaseoli*+PSO+RP (193x$10^6$±0.358 cfu/gm, 91X$10^3$cfu/gmand 170X103cfu/gm,) respectively (Fig. 3). In flowering and final stage also, the maximum microbial load were seen in the plot containing the mixed amendments followed by *R.phaseoli*+RP inoculated plots PSO+RP inoculated pots and RP alone inoculated pots. The least count was observed in the control plots in all stages (Fig 3A,3B,&3C).



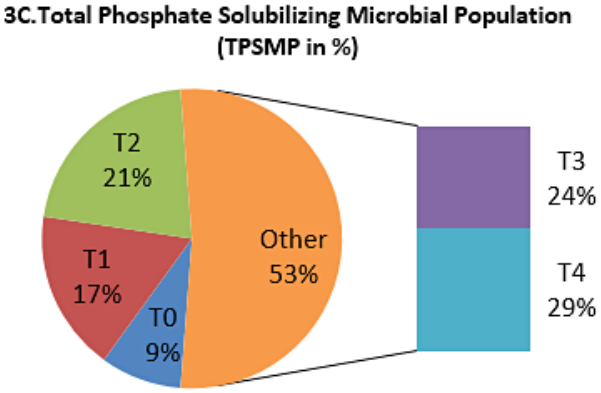



**Fig. 3: Analysis of THBP, THFP and TPSMP at various treatments during pre flowering stage (CFU/ml)**

**Analysis of TPSMP, THFP, and THBP at different flowering stage treatments (CFU/ml)**

During the flowering stage, the T4 treatment contained the highest number of bacteria, fungi and phosphate solubilizing organisms (Fig. 4). This is followed by T3, T2, and T1. The control considered as T0 contained very low levels of this microbial population (Fig. 4A,4B&4C).

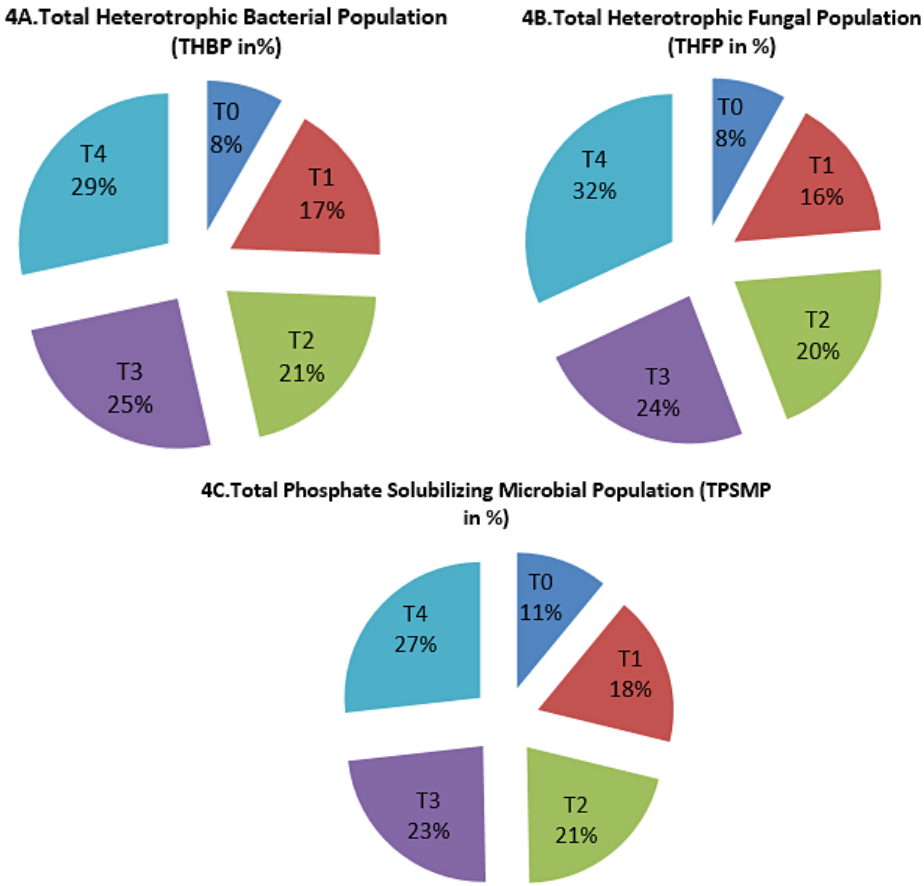



**Fig. 4:Analysis of THBP, THFP and TPSMP at various treatments during flowering stage (CFU/ml)**

**Analysis of TPSMP, THFP, and THBP at different yielding stage treatments (CFU/ml)**

During yielding stage also, bio slurry field containing *R.phaseoli*, PSM and rock phosphate encourages more bacterial ($198X10^6$±1.091cfu/ml) fungal ($112X10^3$±1.234cfu/ml) and phosphate solubilizing microorganisms ($193X10^3$±1.076cfu/ml) populations (Fig. 5). The least counting was observed in control field. In all plots, this microbial population gradually improved from the initial stage and achieved good growth during yielding.

The aforementioned findings demonstrated a significant rise in THBP, THFP, and TPSMP loads from the first to the latter stages of soil analysis. In the *R.phaseoli*+PSO+RPapplied field, a sharp rise in THBP, THFP, and TPSMP was also noted.

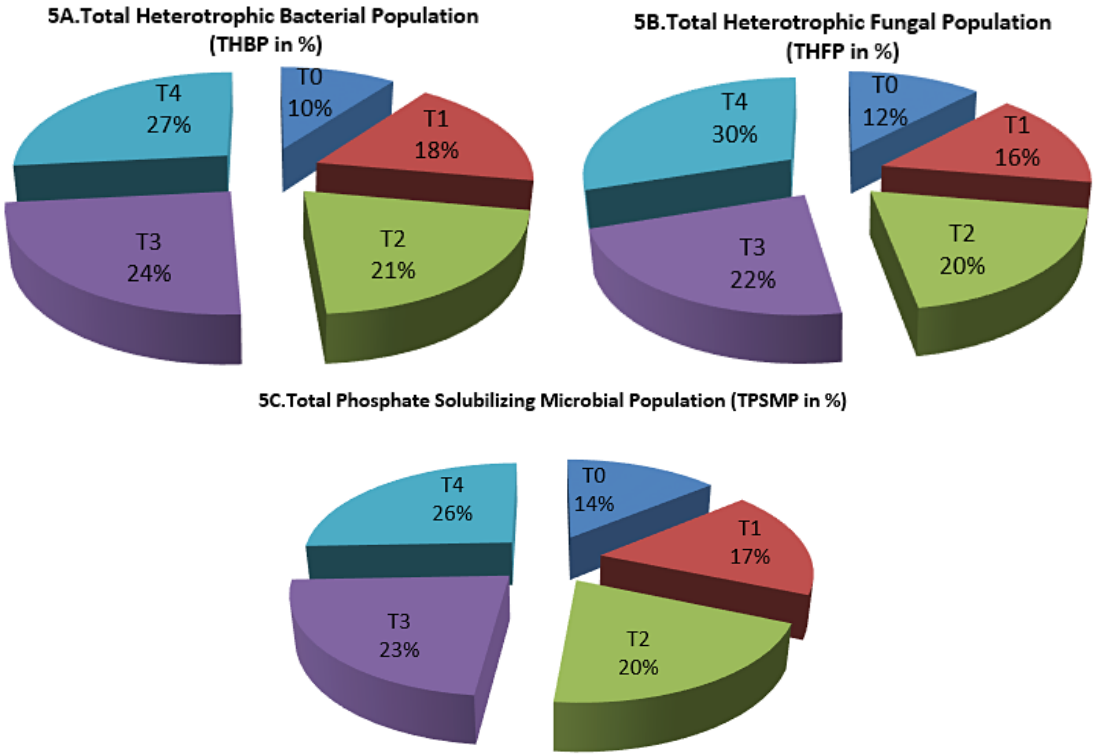



**Fig. 5:Analysis of THBP, THFP and TPSMP at various treatments during yielding stage (CFU/ml)**

**NPK Utility**

The soil's NPK content was measured both before and after manures were applied. A noticeable drop in the initial to final NPK concentration showed that plants were using a significant amount of NPK in the soil for growth and yield (Fig 6). The experimental plant's morphological and biochemical characteristics were measured at various growth stages and recorded. Plants grown in pots treated along rock phosphate with Phosphate Solubilizing Organisms and enriched biodigested slurry along with *R. phaseoli* than others showed a more marked rise in height and dry matter content.

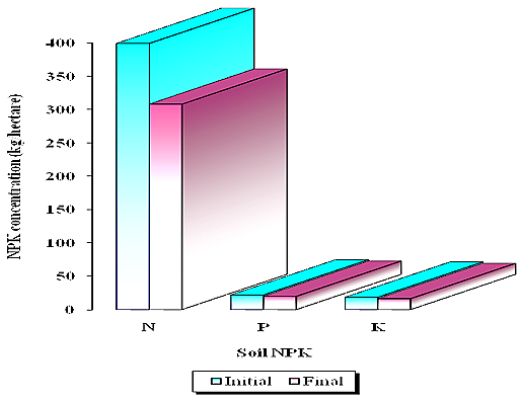



**Fig. 6: Soil NPK content**

**Biodigested Manurial Sources' Impact During the Pre-Flowering Stage**

In pre flowering stage the root length, shoot length, total height, wet weight, dry weight and chlorophyll content were more in the plants cultivated the plots containing *R.phaseoli*+PSO+RP as biodigested organic manure compare to other biodigested organic amendments (Table 4).

**Table 4: Influence of manurial sources on the morphological parameters and chlorophyll content of P.vulgaris grown at pre-flowering stage**

| Manurial sources | Pre-flowering stage | | | | | |
|---|---|---|---|---|---|---|
| | Root length(cm) | Shoot length(cm) | Total height(cm) | Wet weight(g) | Dry weight(g) | Chlorophyll content(mg/lit) |
| T0 | 3.9±0.190 | 5.0±0.289 | 8.9±0.557 | 5.9±0.100 | 0.760±0.110 | 0.140±0.109 |
| T1 | 4.2±0.150 | 5.1±0.793 | 9.3±0.656 | 6.1±0.360 | 0.680±0.014 | 0.142±0.001 |
| T2 | 4.5±0.193 | 5.3±0.468 | 9.8±0.917 | 6.2±0.017 | 0.660±0.105 | 0.152±1.009 |
| T3 | 4.7±1.265 | 6.2±0.036 | 10.9±1.014 | 6.5±0.014 | 0.640±1.010 | 0.158±0.108 |
| T4 | 5.3±0.070 | 6.8±0.610 | 12.1±1.058 | 6.8±0.155 | 0.720±1.006 | 0.182±0.014 |

Each value is expressed as Mean±Standard Deviation.

**Manurial Sources' Influence during the Blossoming Stage**

During the blooming stage, pots treated with *R.phaseoli*+PSO+RP showed the highest chlorophyll content (23.6±0.265/lit), dry weight (9.1±0.265g), and wet weight (9.881±0.055g). In the *R.phaseoli*+PSO+RP applied field, the longest shoot length (34.20.854cm), total height (45.41.637cm), and root length (11.20.200cm) were also noted. (Table 5).

**Table 5: Influence of manurial sources on the morphological parameters and chlorophyll content of *P.vulgaris* grown at flowering stage.**

| Manurial sources | Flowering stage | | | | | |
|---|---|---|---|---|---|---|
| | Root length(cm) | Shoot length(cm) | Total height (cm) | Wet weight (g) | Dry weight(g) | Chlorophyll content(mg/lit) |
| T0 | 8.8±0.080 | 15.3±0.265 | 24.1±0.964 | 4.553±0.056 | 0.491±0.008 | 0.426±0.007 |
| T1 | 7.4±0.361 | 21.1±0.300 | 28.5±0.656 | 7.320±0.019 | 0.572±0.007 | 0.482±0.015 |
| T2 | 9.1±0.265 | 23.6±0.265 | 32.7±0.656 | 7.381±0.016 | 0.714±0.005 | 0.526±0.006 |
| T3 | 9.8±0.700 | 25.4±0.872 | 35.2±0.654 | 9.881±0.055 | 9.1±0.265 | 0.36±0.265 |
| T4 | 11.2±0.200 | 34.2±0.854 | 45.4±1.637 | 9.433±0.014 | 0.618±0.002 | 0.448±0.002 |

Each value is expressed as Mean±Standard Deviation.

**Manurial Sources' Influence at the end Stage**

During the last phase of field plant examination, the *R.phaseoli*+PSO+RP treated plots showed the longest shoot length (40.1±0.625 cm), the wet weight (18.012±0.024g) and dry weight (0.813±0.012g), the longest root length (13.4±1.058cm), and the total height (53.5±0.755cm) of the plants. (Table 6).

**Impact of Manurial Sources on the NPK Content in Plants Cultivated at Various Growth Stages**

Because these nutrients are used to boost the production of the product, *P. vulgaries* plants' NPK content increased from the seedling stage (Table 7) to the flowering stage and then decreased in the last stage of growth. (Table 8).

**Table 6: Influence of manurial sources at final stage**

| Manurial sources | Final stage | | | | | |
|---|---|---|---|---|---|---|
| | Root length (cm) | Shoot length(cm) | Total height(cm) | Wet weight(g) | Dry weight(g) | Chlorophyll content(mg/lit) |
| T0 | 10.4±0.458 | 18.6±0.557 | 29.0±1.039 | 6.21±0.046 | 0.52±0.006 | 0.397±0.009 |
| T1 | 10.2±1.200 | 27.6±0.917 | 37.8±0.800 | 14.374±1.151 | 0.721±0.017 | 0.363±0.015 |
| T2 | 11.5±0.700 | 28.4±0.755 | 39.9±0.529 | 12.637±0.012 | 0.807±0.010 | 0.472±0.011 |
| T3 | 12.1±0.361 | 29.2±0.872 | 41.3±1.153 | 9.610±0.019 | 0.473±0.007 | 0.394±0.005 |
| T4 | 13.4±0.608 | 40.1±0.625 | 53.5±0.755 | 18.012±0.024 | 0.813±0.012 | 0.353±0.012 |

Each value is expressed as Mean±Standard Deviation

**Table 7:NPK content at seedling stage**

| Stage | N(%) | P(%) | K(%) |
|---|---|---|---|
| Seedling stage | 1.262±2.608 | 0.28±0.008 | 0.26±0.609 |

Values in Mean±Standard Deviation

**Table 8: Effect of manurial sources on the NPK content in P .vulgaries grown at different stages of growth.**

| Manurial sources | Pre-flowering (%) | | | Flowering (%) | | | Final (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | N | P | K | N | P | K |
| T0 | 2.126±0.018 | 0.180±0.002 | 0.32±0.010 | 2.760±0.029 | 0.32±0.010 | 0.30±0.020 | 2.4±0.100 | 0.28±0.026 | 0.28±0.010 |
| T1 | 1.898±0.026 | 0.174±0.004 | 0.32±0.017 | 2.868±0.048 | 0.38±0.010 | 0.28±0.017 | 1.8±0.100 | 0.26±0.017 | 0.26±0.906 |
| T2 | 2.126±0.009 | 0.178±0.005 | 0.28±0.010 | 3.000±0.065 | 0.40±0.026 | 0.28±0.026 | 2.8±0.264 | 0.38±0.026 | 0.22±0.026 |
| T3 | 1.926±0.013 | 0.168±0.007 | 0.34±0.017 | 2.768±0.017 | 0.39±0.020 | 0.29±0.017 | 1.9±0.173 | 0.28±0.017 | 0.24±0.017 |
| T4 | 2.826±0.037 | 0.192±0.004 | 0.38±0.017 | 3.845±0.014 | 0.48±0.017 | 0.32±0.010 | 2.8±0.100 | 0.39±0.010 | 0.28±0.020 |

Values in Mean±Standard Deviation

**Nodulation of P.vulgaries in Different Amendments**

Inoculation with *R.phaseoli*+PSO+RP treated pots (T4) recorded the maximum number of nodules(36) at final stage (Fig.7) and the weight of the nodule was 0.039g also showed high germination percentage. It was followed by T3, T2, T1. The control showed poor nodule formation and germination percentage (Table 9).

**Table 9: Influence of R.phaseoli, PSO with RP on nodulation of P.vulgaris**

| Treatments | Germination (%) | Weight of nodules(g) |
|---|---|---|
| T0 | 68.7 | 0.039 |
| T1 | 69.5 | 0.086 |
| T2 | 70.1 | 0.104 |
| T3 | 72.6 | 0.211 |
| T4 | 74.8 | 0.268 |

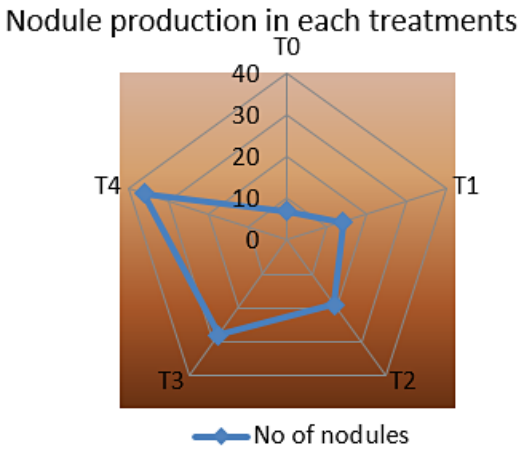



**Fig. 7: Nodule formation in various treatments**

**Impact of Manurial Sources that have been Biodigested on Yield**

The highest recorded cumulative output of *P. vulgaris* was found in the biodigested poultry droppings slurry enhanced with phosphate and phosphate-solubilizing organisms, as well as *R.phaseoli* (2.435 tons/hectare) followed by T3 treatment containing the applied RP with *R.phaseoli* (1.890tons/hec), T2 treatment holed PSM and RP (1.266tons/hec), T1(1.101tons/hec), and finally control (0.821 tons/hectare).Therefore the biodigested slurries obtained from biogas plant enriched with the rock phosphate and phosphate solubilizing organisms gave better yield than the biodigested slurries applied without enrichment (Fig. 8).

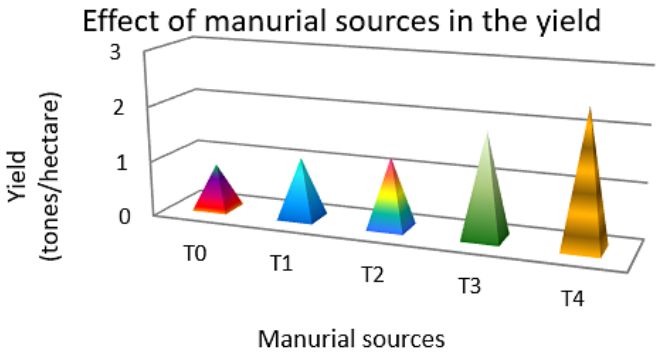



**Fig. 8: Effect of manurial sources in the yield**

**Least Significant Difference Analysis**

**Table 10: LSD in Dry weight of Phaseolus vulgaries during preflowering*, flowering* and final* stages.**

| Manurial sources | Preflowering Stage* | | | |
|---|---|---|---|---|
| | Dry weight (g) | $S.E_x$ | Difference from Control | LSD at 5% |
| To | 0.760 | 0.010 | 0.18 | 0.0196 |
| T1 | 0.680 | 0.011 | 0.10 | 0.0216 |
| T2 | 0.660 | 0.005 | 0.13 | 0.0098 |
| T3 | 0.640 | 0.005 | 0.12 | 0.0098 |
| T4 | 0.720 | 0.10 | - | 0.0059 |
| **Manurial sources** | **Flowering Stage*** | | | |
| | **Dry weight (g)** | **$S.E_x$** | **Difference from Control** | **LSD at 5%** |
| To | 0.491 | 0.008 | - | - |
| T1 | 0.572 | 0.007 | 0.327 | 0.0118 |
| T2 | 0.714 | 0.005 | 0.223 | 0.0098 |
| T3 | 0.900 | 0.007 | 0.081 | 0.0137 |
| T4 | 0.618 | 0.002 | 0.127 | 0.0039 |
| **Manurial sources** | **Final Stage*** | | | |
| | **Dry weight (g)** | **$S.E_x$** | **Difference from Control** | **LSD at 5%** |
| To | 0.52 | 0.006 | - | 0.0118 |
| T1 | 0.72 | 0.017 | 0.201 | 0.0333 |
| T2 | 0.80 | 0.011 | 0.287 | 0.0215 |
| T3 | 0.47 | 0.007 | - | 0.0159 |
| T4 | 0.81 | 0.012 | 0.293 | 0.0236 |

**Conclusion**

During the analysis of Dry weight the difference between control and different treatments included that all the given manurial treatments are highly significant in preflowering stage*, flowering stage*and Final stage* (Table 10).

**Discussion**

In order to increase crop productivity, alternative fertilizer sources have to be considered due to the sharp increase in the price of chemicals and the associated health risks.[24] As a facet in solving this problem, in this study, PSO, *Rhizobium* spp., Rockphosphate (T4), and T3-treated plants produced the highest germination percentages, at 74.8 and 72.6, respectively (Table 9). More nodules (T4–36 numbers) formed after application with a mixed format, followed by T3, T2, and T1 (Fig. 7). The lowest quantity of nodule development seen in the control group. Comparable outcomes have been observed for the nodule's fresh and dry weights.

Digested biogas slurry has a significant impact on crop output when compared to mineral fertilizers, according to studies done by a number of researchers[25,26,27,28,29] Pugesgaard *et al.* (2014)[30] prioritized agricultural feedstock such as grass-clover over maize. Additionally, cow dung and poultry litter are regarded as valuable feedstock for crop development.[31,32,33] According to Govasmark *et al.* (2011)[34] and Heviankova *et al.* (2013),[35] harmful microorganisms and heavy metals are thought to be obtained through the post-digestion of agricultural waste, such as sewage sludge. Therefore, in order to manage the sludge, it must be applied to agricultural areas as biofertilizer.[36,37] Similarly, the slurry that had been resting was mixed fully with the agricultural field after biogas was produced from the chicken farm's excrement.

The necessary anaerobic digestion byproduct often disregarded, bio-slurry has a high nutrient content and has no negative environmental effects.[38] In a similar vein, the biodigested slurry that is administered to every trial field is rich in nutrients, which not only promote plant development and yield but also the steady growth of microorganisms and other beneficial soil fauna. This is accurate, as the number of microorganisms in the control field was larger during the yielding stage than during the first stage despite no additional fertilizer being added.

The advantages of organic farming in developed to developing countries were described by Stockdale (2001).[39] These benefits included increased crop yield without an excessive reliance on expensive inputs, biodiversity enhancement, environmental protection, and reduced energy use and $CO_2$ emissions. According to extensive study, earthworm activity is higher in an organically managed field than in a farming system that uses chemicals for treatment.[40] The output of crops like rice (15.7%), wheat (8.9%), cotton (6.5%), and maize (15.2%) increased as a result of the use of synthetic fertilizer combined with bioslurry.[41] In the same way, compared to other treatments, the biodigested slurry including RP, PSO, and *R.phaseoli* produced superior results in the T4 treatment.

Additionally, compared to non-enriched control pots, the biodigested slurry enhanced with Rock Phosphate and Phosphate Solubilizing Organisms (RP+PSO) produced greater results next to T4. According to Liu *et al.* (2009)[42] soil productivity is reduced if solely synthetic fertilizers are used. A P dressing was found to enhance nitrogen fixation in legumes by Albrecht *et al.* (1948)[43] most likely as a result of enhanced nodulation and root growth. In comparison to the control, PSO and *Rhizobium* that were infused with RP (T4) exhibited a notable increase in shoot length. As the plant grew older, the fresh and dry weight of the shoot and root rose, reaching its maximum in the T4 treatment. There was a big difference between this treatment and the others. Due to the high expense of chemical fertilizers, it was necessary to discover an alternative. Often, using bioslurry in conjunction with synthetic fertilizers produced higher yields than using bioslurry alone.[44]

The distribution of *Rhizobium* to increase the nitrogen requirement of growing legumes was established in the experiment of inoculating the leguminous plants with VA mycorrhizal fungi and effective strains of *Rhizobium* and phosphate solubilizing organisms separately and in dual form. This increased nodulation, nitrogen fixation, and accumulation.[45] Like that, in this observation also, the plots enriched with *R.phaseous* and PSO either in a mixed form or in separate form gave comparatively good results than control. A substantial population of this agriculturally significant *Rhizobium* species was found when it was cultured using organic manures.

After conducting experiments, Nagarajan and Balachandar (2001)[46] found that, out of all the organic amendments utilized, the biodigested slurry from the biogas plant that had been inoculated with *Rhizobium* produced the notable physiological parameter, and grain yield for both black and green gram, respectively. The organic manure made from biogas slurry and RP provided good morphological and biochemical observations of the experimental plants for this work as well. Three studies have found that applying RP along with PSO improved the amount of P that was accessible to the plants.[47,48] The NPK content of *P. vulgaris*, shoots and roots was higher in plants injected with PSO and *Rhizobium* in addition to RP than in plants inoculated with RP alone (Table 8).

The increased plant growth and uptake of P may be the result of soil microbes producing CO2. According to Jurinak (1986).[49] $CO_2$ accelerated

the breakdown of calcium apatite. Under most circumstances, the dissociation of carbonic acid—which is mostly produced from $CO_2$ as a result of biological respiration—is the main source of protons on the rhizosphere. During the crop growing phase, the PSO population was generally greater in the inoculation treatments compared to the uninoculated treatments (Table 3). When PSO and RP (T4) were applied, the largest number of organisms was recorded. *Rhizobium* sp. population was highest in treatment T3. Up until the very end, the infected organisms proliferated greatly, and the NPK content, they began to decrease during the harvest phase. There was a decrease in harvest time and a proliferation of inoculated PSO and *Rhizobium* up to the final stage.[50] The statistics unequivocally demonstrated that accessible P was more important to the population of potential PSOs than other factors. It may be concluded that nodulation and nitrogen fixation in legumes can be significantly impacted by PSO, *Rhizobium*, and rock phosphate.

**Conclusion**

In both developed and emerging nations, organic farming is necessary for ecological and economic sustainability as well as a stable means of subsistence. It produces high-quality food without degrading the environment or the condition of the soil. Digested Biogas Slurry has an average nutritional makeup of 1.262%, 0.28%, and 0.26%. These biodigested slurries improve the soil's endophytic bacteria, which increases the amount of organic matter produced. Even in the control field, the applied bioslurry in the current study progressively increased the microbial development from the initial stage to the yielding stage. In addition to digested biogas slurry, there is an increase in organic matter in the soil, which is necessary for plant growth. Because poultry manure contains more nutrients than the slurry made from cow dung, the biogas slurry made from poultry litter produces more yields in applied field. The intake capacity of crops, vegetables, and fodders remains a crucial production parameter despite the soil's increasing nutrient content. Utilizing digested biogas slurry (DBGS) can cut down on the usage of synthetic or chemical fertilizers by 50%. Both biogas residue and slurry are excellent for maintaining and enhancing soil fertility, as well as for improving crop productivity and quality. When bio-slurry is used in the field, production quality is improved and chemical fertilizer consumption costs are decreased, enhancing marketability and contributing to farmers' economic prosperity. Therefore, using biogas slurry can improve the field's sustainability while also lowering the expense of chemical fertilizer.

**Authors' Contributions**

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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**Conflicts of Interest**

The authors declare that there is no conflict of interest.

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