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Research Article

# Effect of Fertilizer Sources on Soil Organic Matter and Soil Microbial Population in Broccoli Field

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## Abstract

Soil microbes show positive ecological interactions which promote nutrient recycling, decomposition and plant growth. To study the effect of different fertilizer sources on soil organic matter and soil microbial population in broccoli field, an experiment was conducted at Agriculture and Forestry University, Chitwan, Nepal from September 2015 to February 2016. The research consisted of ten treatments viz; recommended NPK fertilizer, Farmyard manure (FYM), Vermicompost (VC), Cow urine (CU), Bio organic fertilizer (BOF), (NPK 50% + FYM 50%), (FYM 50% + CU 50%), (FYM 50% + VC 50%), (FYM 50% + BOF 50%) and (25% FYM + 25% VC+ 25% CU+ 25% BOF) in randomized complete block design replicated three times. The study revealed that highest soil organic matter was recorded in FYM (50%) + vermicompost (50%) treatment (2.85%) which remained statistically similar with all other organic treatments. The recommended NPK treatment had significantly lowest SOM (2.06%). The highest bacteria population (43.6 x $10^7$ cfu/g) and highest fungi population (11.0 x $10^6$ cfu/g) were observed in treatment consisting all organic fertilizer sources (25% FYM + 25% VC+ 25% CU+ 25% BOF). In recommended NPK treatment, the bacteria and fungi population were 4.10 x $10^7$ cfu/g and 7.1 x $10^6$ cfu/g in $10^{-6}$ dilution respectively. But, in the treatments consisting organic fertilizer sources, bacteria population ranged from 2.93 x $10^7$ cfu/g to 43.6 x $10^7$ cfu/g and fungi population ranged from 7.33 x $10^5$ cfu/g to 11.0 x $10^6$ cfu/g in the same dilution respectively. Positive regression relation was also observed in between soil organic matter and soil microbes. Therefore, organic fertilizer sources supported for soil organic matter enrichment and promotion of bacteria and fungi population in the soil in comparison to chemical fertilizers.

**Keywords:** Soil Microbes; Soil Organic Matter; Colony Forming Units; Organic Fertilizer

## Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is one of the important vegetables belonging to family Brassicaceae.

It contains abundant vitamins and minerals such as vitamin A and C, carotenoids, fiber, calcium, and folic acid [1]. Broccoli and other brassica vegetables have high content of glucosinolates which has anti-cancer properties [2]. Soil hosts bacteria, fungi, other microbes and animals. Numbers of microorganism may vary in and between different soil types and conditions, with bacteria being the most numerous. Bacterial counts in different soils ranged from 4 x $10^6$ to 2 x $10^9$ per gram of dry soil [3]. Similarly, soil fungi are also most abundant group of soil microorganisms, on a mass basis, and their biomass ranges from 100 to 1500 grams/$m^2$ of soil [4]. Growth of microbes and their action on soils are dependent on the interaction between plant species and soil [5]. Bacterial community composition results from the interaction between soil type, plant species and its rhizosphere localization [6]. Soil microbes, mostly fungi and bacteria are active participants in the ecosystems and perform organic matter decomposition, liberating and recycling chemical nutrients, formation of soil aggregates, detoxification of organic toxicants, promotion of plant growth etc. [7,8]. They show the positive ecological interactions that promote plant growth. The growth of soil microbes is usually carbon limited, so the high amounts of sugars, amino acids and organic acids that plants deposit into the rhizosphere represent a valuable nutrition source [9].

The increasing awareness of the harmful effects of indiscriminate use of chemical fertilizers and pesticides has led to the adoption of organic fertilizers and manures. Organic manure can serve as alternative practice to mineral fertilizers for improving soil structure [10] and microbial biomass [11]. It is also a source of food for the innumerable number of microorganisms and creatures like earthworm which break down the organic matter into plant available nutrients, which are easily absorbed by the plants. Soil organic matter improves soil texture, increases ion exchange capacity of soil, increases soil microbial populations and activity, improves moisture-holding capacity of the soil and enhances soil fertility [12]. Soil organic matter consists of broad groups of substances, often called pools that vary in their rates of decomposition and functions. One of the most important pools of organic matter is the microbial biomass. Although the size of the microbial biomass is relatively small, the nutrients within this pool are recycled rapidly within the soil profile, perhaps 8 to 10 times per year [13]. The microbial biomass is a relatively available reservoir of plant nutrients such as nitrogen and phosphorus [14].

Therefore, an experiment was carried out to find the effects of different fertilizer sources on soil organic matter and microbial population in a broccoli field at Agriculture and Forestry University, Rampur, Chitwan during 2015/16.

## Methodology

The study was conducted in Agriculture and Forestry University, Rampur, Chitwan (from September 2015 to February 2016) in randomized complete block design (RCBD) with ten treatments (Table 1) and was replicated thrice. The physico-chemical properties of field soil (30 cm depth) before the experimentation were as presented in the table 2. Amounts of fertilizers for each treatments were calculated based on recommended dose of fertilizer for broccoli [15]. Urea was applied in two equal splits first as basal and second as top dress at 30 days after transplanting (DAT). In case of cow urine, at first, it was left for two weeks for fermentation and then applied over the soil surface in 7 days interval diluted with water in the ratio of 1:2 [16,17]. Each plot had 5.4m$^2$ area with 20 plants planted in 60 x 45 crop spacing. Likewise, soil organic matter (SOM) was measured for each plot after the crop harvest. Details of experimental field's soil and nutrient contents of different organic fertilizers used in experiment are given in table 2 and 3 respectively.

To determine the soil microbial population, serial dilution method was used. This method is used for plating and enumerating live microorganisms in a given population. In this technique, plating was done and determination of the total number of bacteria and fungi was done in the original solution by counting the number of colony forming units (cfu) and comparing them to the dilution factor [20]. Each CFU represents a bacterium that was present in the diluted sample. For bacteria, Nutrient Agar media was used with pH 7.0 and Czapek dox media with pH 7.3 was used for fungi. Compositions used to prepare these media for bacteria and fungi are given in the table 4 and 5 respectively.

| Treatment symbols and combinations | Amount of fertilizers* |
|---|---|
| T1: Recommended NPK | 137g urea, 207.67g DAP, 70.76g potash |
| T2: NPK (50%) + FYM (50%) | 68.5g urea, 103.5g DAP, 35g potash, 20 Kg FYM |
| T3: FYM | 40 Kg FYM |
| T4: Vermicompost | 11 Kg vermicompost |
| T5: Cow urine | 20L. cow urine |
| T6: Bio organic fertilizer (BOF) | 9 Kg BOF |
| T7: 50% FYM + 50% cow urine | 20 Kg FYM, 10L. cow urine |
| T8: 50% FYM + 50% vermicompost | 20 Kg FYM, 5.5 Kg vermicompost |
| T9: 50% FYM + 50% BOF | 20 Kg FYM, 4.5 Kg BOF |
| T10: 25% FYM + 25% Vermicompost + 25% cow urine + 25% BOF | 10 Kg FYM, 2.75 Kg vermicompost, 2.25 Kg BOF, 0.83L. cow urine |

**Table 1:** Treatment symbols, combinations and amounts of fertilizers in each treatment.

Note: *Amounts of fertilizers per plot, urea was applied in two equal splits; first half as basal and second half as top dress at 30 days after transplanting.

| S.N. | Properties | Average content | Category | Reference |
|---|---|---|---|---|
| 1. | Physical properties | | | |
| | Sand (%) | 72.1 | - | |
| | Silt (%) | 22.8 | - | |
| | Clay (%) | 5.1 | - | |
| 2. | Textural class (USDA) | - | Sandy loam | [18] |
| 3. | Bulk density | 1.41 | | |
| 4. | Chemical properties | | | [19] |
| | Soil pH | 6.4 | Towards neutral | |
| | Soil organic matter (%) | 2.65 | Medium | |
| | Total nitrogen (%) | 0.14 | Medium | |
| | Available phosphorus (Kg/ha) | 17.33 | Medium | |
| | Available potassium (Kg/ha) | 160.8 | Medium | |

**Table 2:** Physico-chemical properties of the soil of the experimental site in Chitwan, Nepal, 2015/16.

| S.N. | Organic fertilizers | Nitrogen (%) | Phosphorus (%) | Potash (%) |
|---|---|---|---|---|
| 1 | FYM | 0.65 | 0.51 | 1.05 |
| 2 | Vermicompost | 1.70 | 0.65 | 2.66 |
| 3 | Cow urine | 0.90 | 0.45 | 1.95 |
| 4 | BOF | 2.0 | 1.0 | 1.2 |

**Table 3:** Nutrient content of different organic fertilizer sources used in experiment.

| Composition | Amount |
|---|---|
| Peptone | 5.0g |
| Beef extract | 3.0g |
| NaCl | 5.0g |
| Agar | 15g |
| Distilled water | 1000 ml |
| Yeast extract | 1.5g |

**Table 4:** The composition of Nutrient Agar Media [21].

| Composition | Amount |
|---|---|
| Sodium Nitrate | 2g |
| Dipotassium hydrogen phosphate | 1.0g |
| Magnesium sulphate | 0.5g |
| Potassium chloride | 0.5g |
| Ferrous sulphate | 0.01g |
| Sucrose | 30g |
| Agar | 15g |
| Distilled water | 1000 ml |

**Table 5:** The composition of Czapek dox media [22].

After the respective incubation period, bacterial and fungal colonies in each plate were counted and microbial populations was determined per gram soil by multiplying the average count by dilution factor. The colony are formed by the viable microorganisms present in the soil sample. These are called Colony Forming Units (cfu). Fungal or Bacterial population or population in the given original sample is represented by no of cfu per ml of sample as given in the following formula [23]:

$$\frac{\text{Number of cfu}}{\text{Volume plated (ml)} \times \text{Dilution Factor}}$$

Therefore, Bacterial population per gram of the soil= number of bacterial colonies × $10^6$

Fungal population per gram of the soil= number of fungal colonies × $10^5$.

### Data analysis

The data collected in the experiment were statistically analyzed with GEN-STAT Version 4.0 statistical software program. Analysis of variance (ANOVA) was done on every measured parameter to determine the significance of differences between means of treatments. Means for each parameter were separated by the Duncan's multiple range test (DMRT) and least significant difference (LSD) at $P \leq 0.05$. Similarly, for the bacterial and fungal population, counting of colony forming units and calculations were carried for the comparison among the treatments.

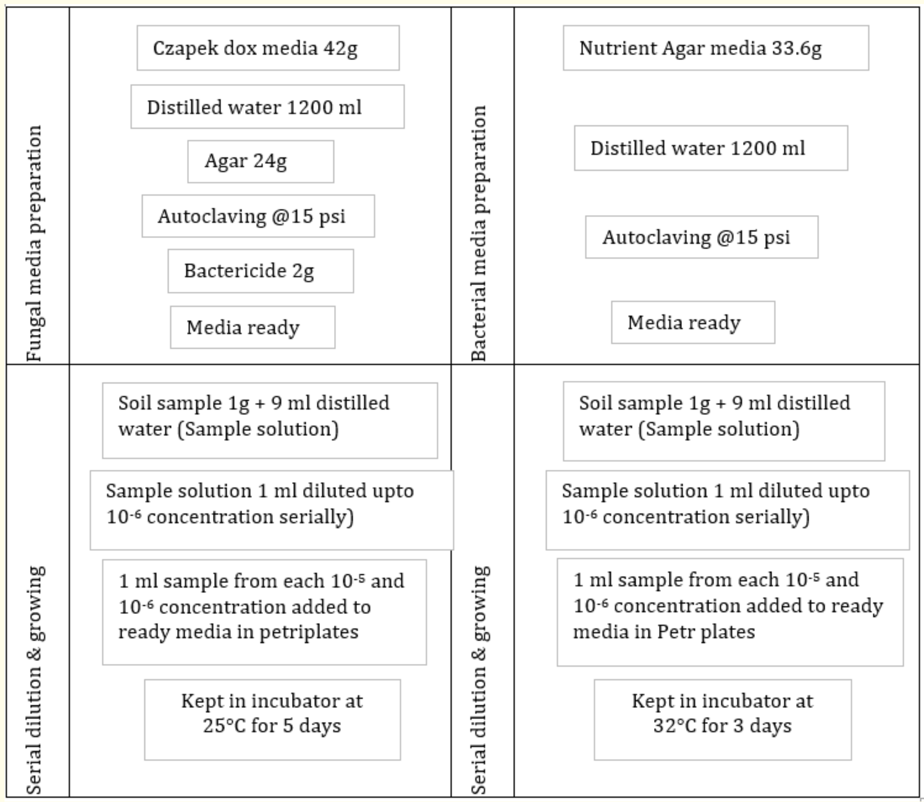


**Figure 1:** Schematic diagram for steps in media preparation and serial dilution and culturing procedure.

## Results and Discussion

### Soil organic matter

The SOM is indicator of soil quality and fertility [24]. It is one of three soil components that are crucial for its physicochemical properties, such as its sorptive and buffer abilities as well as its biodiversity and biological activity [25]. This experiment showed that soil organic matter was found significantly influenced by the different fertilizer sources (Table 6). The highest SOM was recorded in FYM (50%) + vermicompost (50%) treatment (2.85%). It remained statistically similar with all other treatments with sole and combined organic sources and statistically different with recommended NPK treatment (2.06%). Concerning the role of FYM, due to its high level of organic C, it was responsible for increasing soil organic carbon/matter in a short term [26]. This might be due to similar increase in soil reactions among all pure organic treatments as compared to other treatments consisting chemical fertilizer. Also, an increase in soil reactions due to the application of organic fertilizer and manure was stated by Petek [27]. The treatments FYM, cow urine, vermicompost and BOF showed SOM as 2.66%, 2.45%, 2.72% and 2.77% respectively whereas combination of all these organic sources resulted SOM as 2.68%. Treatments with sole application of the organic sources and that with combination of organic sources had no significant differences in SOM. The recommended NPK treatment had significantly lowest SOM (2.06%). Here, lower SOM was recorded in the treatments with inorganic NPK application. Similar studies indicated that chemical fertilizer reduces SOM stocks because it enhances soil organic matter mineralization [28,29]. This revealed that FYM, vermicompost, bio-organic fertilizer, cow urine and their combinations had positive effect on SOM. Application of organic fertilizers with or without chemical fertilizer significantly increased soil organic matter but application of inorganic fertilizer alone had no effects [30,31]. Similarly, an increase in SOM due to application of either FYM or FYM + cow urine was also reported by Veeresha., *et al* [32]. Such findings were also reported by Piaszczyk., *et al.* [33] in forest nursery soil. They found increase in organic matter by 33% - 40% in organic fertilizer applied soils in relation to the control soils. Likewise, roles of bio-fertilizers to maintain or increase the content of organic matter and improve soil fertility in arable soils

| Treatments | Soil organic matter (%) |
|---|---|
| T1: Recommended NPK | 2.06c |
| T2: NPK (50%) + FYM (50%) | 2.29bc |
| T3: FYM | 2.66ab |
| T4: Vermicompost | 2.72a |
| T5: Cow urine | 2.45ab |
| T6: Bio organic fertilizer (BOF) | 2.77a |
| T7: 50% FYM + 50% cow urine | 2.70a |
| T8: 50% FYM + 50% vermicompost | 2.85a |
| T9: 50% FYM + 50% BOF | 2.83a |
| T10: 25% FYM + 25% Vermicompost + 25% cow urine + 25% BOF | 2.68ab |
| LSD (0.05) | 0.37 |
| SEm (±) | 0.12 |
| CV (%) | 8.2 |
| P value | ** |

**Table 6:** Effect of different fertilizer sources on soil organic matter in Chitwan, Nepal, 2015/16.

Note: Means followed by the same letter(s) in the same column are not significantly different at 5% level of significance by DMRT.

**Represents significant at 1 % level of significance.

stated by Dinesh., *et al.* 2010 [34].

## Soil microbial population

Counting the CFU and simultaneously calculating the microbial population in the given soil sample revealed that both bacteria and fungi population ranged relatively higher in the treatments with sole or combined organic fertilizer sources than in recommended NPK except bacteria population in FYM (50%) + cow urine (50%) treatment (Table 7 and 8). The highest bacteria population (37.6 x $10^6$ cfu/g and 43.6 x $10^7$ cfu/g in $10^{-5}$ and $10^{-6}$ dilutions respectively) was observed in the treatment combining all four organic fertilizer sources i.e. FYM (25%) + vermicompost (25%) + Cow Urine (25%) + BOF (25%) followed by BOF (17.3 x $10^6$ cfu/g and 13.7 x $10^7$ cfu/g in $10^{-5}$ and $10^{-6}$ dilutions respectively) (Table 7). Similar trend was observed in case of fungi population. Maximum fungi population observed was 10.0 x $10^5$ cfu/g and 11.0 x $10^6$ cfu/g (in $10^{-5}$ and $10^{-6}$ dilutions respectively) in the treatment FYM (25%) + vermicompost (25%) + Cow urine (25%) + BOF (25%) followed

| Treatments | 1st Reading (after 24 hrs.) | | 2nd Reading (after 48 hrs.) | |
|---|---|---|---|---|
| | Dilution factor | | Dilution factor | |
| | $10^{-5}$ | $10^{-6}$ | $10^{-5}$ | $10^{-6}$ |
| T1: Recommended NPK | 2.45 x $10^6$ | 2.52 x $10^7$ | 3.21 x $10^6$ | 4.10 x $10^7$ |
| T2: NPK (50 %) + FYM (50 %) | 2.8 x $10^6$ | 2.70 x $10^7$ | 4.27 x $10^6$ | 5.90 x $10^7$ |
| T3: FYM | 2.57 x $10^6$ | 3.13 x $10^7$ | 4.20 x $10^6$ | 6.50 x $10^7$ |
| T4: Vermicompost | 2.20 x $10^6$ | 3.63 x $10^7$ | 3.06 x $10^6$ | 5.17 x $10^7$ |
| T5: Cow urine | 1.67 x $10^6$ | 4.9 x $10^7$ | 3.50 x $10^6$ | 5.67 x $10^7$ |
| T6: Bio organic fertilizer (BOF) | 12.0 x $10^6$ | 7.8 x $10^7$ | 17.3 x $10^6$ | 13.7 x $10^7$ |
| T7: 50% FYM + 50% cow urine | 10.9 x $10^6$ | 1.37 x $10^7$ | 16.2 x $10^6$ | 2.93 x $10^7$ |
| T8: 50% FYM + 50% vermicompost | 5.63 x $10^6$ | 4.9 x $10^7$ | 7.53 x $10^6$ | 6.87 x $10^7$ |
| T9: 50% FYM + 50% BOF | 2.60 x $10^6$ | 5.36 x $10^7$ | 3.23 x $10^6$ | 5.77 x $10^7$ |
| T10: 25% FYM + 25% Vermicompost + 25% cow urine + 25% BOF | 11.1 x $10^6$ | 19.4 x $10^7$ | 37.6 x $10^6$ | 43.6 x $10^7$ |

**Table 7:** Effect of different fertilizer sources on soil bacteria opulation (cfu/g) at Rampur, Chitwan, 2015/16.

by 7.87 x $10^5$ cfu/g and 9.33 x $10^6$ cfu/g (in $10^{-5}$ and $10^{-6}$ dilutions respectively) in vermicompost treatment (Table 8).

| Treatments | 1st Reading (after 48 hrs.) | | 2nd Reading (after 72 hrs.) | |
|---|---|---|---|---|
| | Dilution factor | | Dilution factor | |
| | $10^{-5}$ | $10^{-6}$ | $10^{-5}$ | $10^{-6}$ |
| T1: Recommended NPK | 4.12 x $10^5$ | 5.44 x $10^6$ | 6.19 x $10^5$ | 7.1 x $10^6$ |
| T2: NPK (50%) + FYM (50%) | 4.33 x $10^5$ | 6.14 x $10^6$ | 7.0 x $10^5$ | 8.0 x $10^6$ |
| T3: FYM | 7.33 x $10^5$ | 6.0 x $10^6$ | 8.33 x $10^5$ | 7.33 x $10^6$ |
| T4: Vermicompost | 5.33 x $10^5$ | 6.63 x $10^6$ | 7.87 x $10^5$ | 9.33 x $10^6$ |
| T5: Cow urine | 5.0 x $10^5$ | 5.67 x $10^6$ | 7.0 x $10^5$ | 8.33 x $10^6$ |
| T6: Bio organic fertilizer (BOF) | 5.67 x $10^5$ | 5.33 x $10^6$ | 7.67 x $10^5$ | 8.67 x $10^6$ |
| T7: 50% FYM + 50% cow urine | 4.67 x $10^5$ | 4.33 x $10^6$ | 7.0 x $10^5$ | 7.67 x $10^6$ |
| T8: 50% FYM + 50 % vermicompost | 4.67 x $10^5$ | 4.33 x $10^6$ | 7.33 x $10^5$ | 8.33 x $10^6$ |
| T9: 50% FYM + 50% BOF | 6.67 x $10^5$ | 5.0 x $10^6$ | 9.33 x $10^5$ | 7.67 x $10^6$ |
| T10: 25% FYM + 25% Vermicompost + 25% cow urine + 25% BOF | 7.0 x $10^5$ | 8.33 x $10^6$ | 10.0 x $10^5$ | 11.0 x $10^6$ |

**Table 8:** Effect of different fertilizer sources on soil fungi population (cfu/g) at Rampur, Chitwan, 2015/16.

Regarding the bacteria population (Table 7), applications of FYM and cow urine resulted the population of bacteria to be 6.50 x $10^7$ cfu/g and 5.67 x $10^7$ cfu/g respectively whereas their combined application (FYM 50% + cow urine 50%) resulted the population to be 2.93 x $10^7$ cfu/g which is the lowest value. Similarly, vermicompost and bio-organic fertilizer resulted the bacteria population to be 5.17 x $10^7$ cfu/g and 13.7 x $10^7$ cfu/g respectively. Combined treatments FYM (50%) + Vermicompost (50%) and FYM (50%) + BOF (50%) had bacteria population 6.87 x $10^7$ cfu/g and 5.77 x $10^7$ cfu/g respectively. Regarding the fungi population (Table 8), applications of FYM and cow urine resulted the population of fungi to be 8.33 x $10^6$ cfu/g and 7.33 x $10^6$ cfu/g respectively whereas their combined application (FYM 50% + cow urine 50%) resulted the population to be 7.67 x $10^6$ cfu/g. Similarly, vermicompost and bio-organic fertilizer resulted the fungi population to be 9.33 x $10^6$ cfu/g and 8.67 x $10^6$ cfu/g respectively. Combined treatments FYM (50%) + Vermicompost (50%) and FYM (50%) + BOF (50%) had fungi population 8.33 x $10^6$ cfu/g and 7.67 x $10^6$ cfu/g respectively.

Use of organic matter stimulates growth and development of beneficial microorganisms [35]. Although there are very few studies regarding effect of cow urine on soil microbes, significant increase in soil fungi and bacteria population over the control was reported by Veeresha., *et al.* [32] when applying FYM and cow urine as organic fertilizers. Soil microbial biomass is one of the most sensitive indicators of soil quality changes [36]. Even short-term application of organic manures and bio-fertilizers promoted soil microbial and enzyme activities and these parameters are sensitive enough to detect changes in soil quality [34].

In recommended NPK treatment, the bacterial population was 3.21 x $10^6$ cfu/g and 4.10 x $10^7$ cfu/g in $10^{-5}$ and $10^{-6}$ dilution respectively. But it ranged from 3.06 x $10^6$ cfu/g to 37.6 x $10^6$ cfu/g and 2.93 x $10^7$ cfu/g to 43.6 x $10^7$ cfu/g in $10^{-5}$ and $10^{-6}$ dilutions respectively for the treatments consisting organic fertilizers sources. Similarly, fungi population in recommended NPK treatment was 6.19 x $10^5$ cfu/g and 7.1 x $10^6$ cfu/g in $10^{-5}$ and $10^{-6}$ dilutions respectively. But it ranged from 7.0 x $10^5$ cfu/g to 10.0 x $10^6$ cfu/g and 7.33 x $10^5$ cfu/g to 11.0 x $10^6$ cfu/g in $10^{-5}$ and $10^{-6}$ dilutions respectively for the treatments consisting organic fertilizers sources. This showed that soil microbial population were higher in organic treatments as compared to inorganic treatments. Lower microbial population in the soil might be attributed to the enhanced acidity caused by chemical fertilization which is unfavorable for microbes' growth and development e.g. *Actinomycetes* [37]. Addition of organic amendments such as manures results in increased microbial biomass (soil bacteria and fungi) and higher microbial activity in the soil [38]. Likewise, Shi-wei and Fuzhen [39] emphasized that organic fertilizer sources have greatly increased surface areas providing more microsites for microbial decomposing organisms, and strong adsorption and retention of nutrients thereby supporting for increment of the soil microbes.

Many studies had shown that soil microbial biomass and communities are changed by organic fertilizer sources and these changes may relate to soil organic carbon/matter content [40]. Positive linear regression was found in between SOM and bacteria population ($R^2$ = 0.0342, Figure 2) and that in between SOM and fungi population ($R^2$ = 0.112, Figure 3). A positive linear regression relationship between microbial diversity and soil organic carbon suggested that increase in microbial biomass and functional diversity might be due to increase in carbon availability resulting from manure amendments [41].

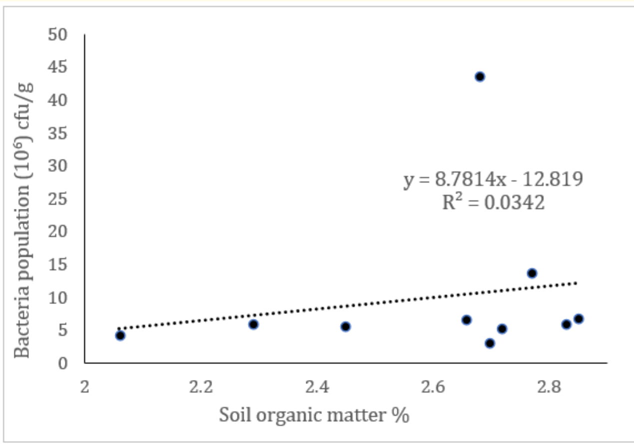


**Figure 2:** Linear regression between soil organic material and bacterial population.

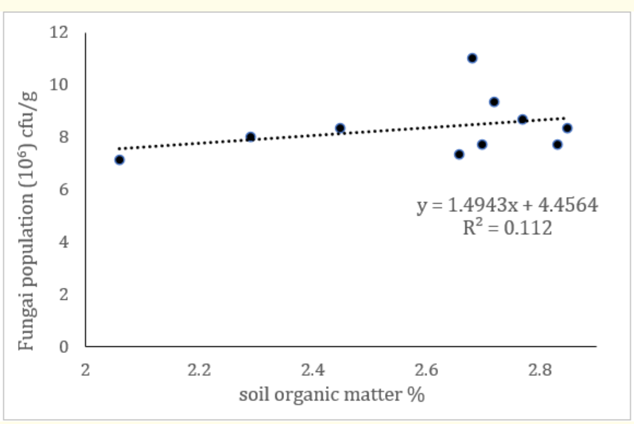


**Figure 3:** Linear regression between soil organic material and fungi population.

## Conclusion

The organic fertilizer sources enriched the organic matter content in the soil thereby enhancing the soil fungi and bacteria population. Combining all the organic sources i.e. farmyard manure (25%) + vermicompost (25%) + bio-organic fertilizer (25%) + cow urine (25%) resulted the maximum population of soil bacteria and fungi and combining farmyard manure 50% + vermicompost 50% ensured the highest organic matter content in the soil.

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