Research article

Enhancing Fermentation of Farmyard Manure Using *Bacillus* sp. in the Mid-hills of Nepal

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Abstract Farmers in the mid-hills of Nepal have been using farmyard manure for maintaining soil fertility for their lands. However, there has been a decrease in production of farmyard manure. Less application of farmyard manure has led to declined soil fertility and reduced nutrient balance. Soil degradation through nutrient depletion is a serious issue. Soils in mid hills have very low nutrients, particularly nitrogen and phosphorus concentration. Sustainability of hill agriculture depends on how farmers use the available resources. Application of incompletely fermented and decomposed farmyard manure could have low nutrient contents, cause health risks as plants can uptake pathogenic bacteria through soil. Also, imbalances in nutrient content of manure mainly nitrogen and phosphorus can pollute water sources through leaching and runoff. An experiment was conducted to determine the action of *Bacillus* sp. as an inoculant for composting. Three different concentrations of *Bacillus* sp. liquid were extracted from locally available rice husk, which were 6.0×10^{10}, 6.0×10^{12}, 6.0×10^{14} cfu mL^{-1}. An experiment was conducted for 60 days comparing samples with and without *Bacillus* sp. liquid. Results showed that *Bacillus* sp. helped in enhancing the fermentation process and better decomposition of organic matter, mineralization and the heat generated during the process helped in the elimination of pathogenic bacteria. Further, a growth experiment was conducted where *Brasicca rapa* was grown on the compost prepared. The results of growth experiment showed application of *Bacillus* sp. is good for development of plants.

Keywords Nepal, mid-hills, farmyard manure, *Bacillus* sp., soil fertility

INTRODUCTION

Farmers in the mid hills of Nepal (Fig. 1) have been using farmyard manure for maintaining soil fertility for their lands. However, there has been decreasing production of farmyard manure. Less application of farmyard manure has led to decline soil fertility and reduce nutrient balance (Regmi et al., 2005). Soil degradation through nutrient depletion is also a serious issue (Lal, 2000). Soils in mid-hills have very low nutrients, especially nitrogen and phosphorus concentration (Shah and Schreier 1991; Brown 1997; Westarp et al., 2004).

Application of farmyard manure, which is not completely fermented, raises health risks as plants can uptake pathogenic bacteria. Also, imbalance in nutrient contents of manure mainly from nitrogen and phosphorus can pollute water sources through leaching and runoff.

Adding of microbial inoculants often referred as effective microorganisms (EM) have been used in composting process. Li et al., (2013) suggested improvement of crop residue composting using *Bacillus* sp. Also, Kuroda et al., (2009) stated better quality compost using the same. Studies have been conducted addressing the use of microbial inoculants with the purpose of accelerating composting and improving the final product (Zeng et al., 2009; Figueiredo et al., 2013). García et al., (2006) observed in their studies that the use of bacterial inoculants (*Bacillus* and actinobacteria) in the composting of vegetable products increased the final humification of the compost and consequentially improved the agricultural quality of the product. Nevertheless, further information about the composting system is necessary.

Therefore, the aim of this study was to assess the effect of *Bacillus* sp. in composting process by evaluating the mineralization and its effect in eliminating pathogenic bacteria.

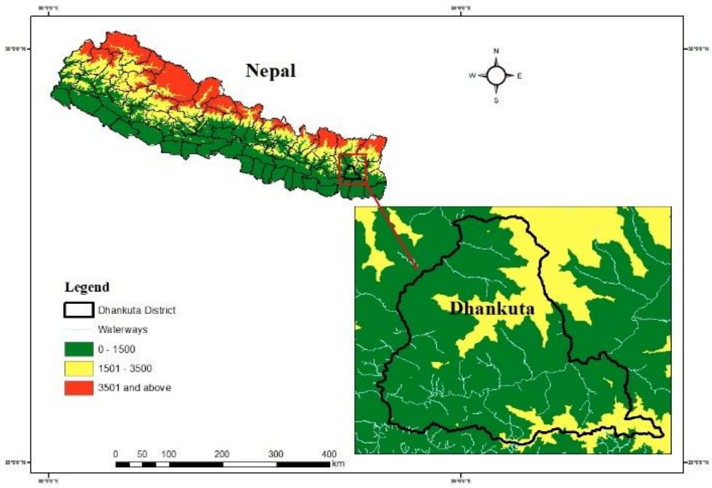

Fig. 1 Digital elevation model map of Nepal and study site

METHODOLOGY

Isolation of *Bacillus* sp.

In this study, *Bacillus* sp. solution was extracted from rice straw and husk. Ten grams of rice husk and straw was added in 90 mL of sterile water and kept overnight in shaker. Serial dilutions were made and inoculated in nutrient agar plates which was incubated for 24 h at 30°C. Serial plate count method was used to determine the number of colonies on nutrient agar. Three different *Bacillus* sp. solutions were made which were 6.0×10^{10}, 6.0×10^{12} and 6.0×10^{14} cfu mL^{-1} was made in nutrient broth. The *Bacillus* sp. solution was made 200 mL for each cfu mL^{-1}.

Sample Preparation

Cow manure and leaves were used in this composting experiment, where 1 kg of cow manure was mixed with 1 kg of leaves to make a total mass of 2 kg. Physical, chemical and microbial experiments were conducted to analyze the initial condition of materials used. Four treatments were made which were Control, Treatment 1, Treatment 2 and Treatment 3 (Table 1). In treatments other than Control, 200 mL of *Bacillus* sp. liquid was added and mixed thoroughly.

Table 1 Preparation of samples for composting experiment

Control	Treatment 1	Treatment 2	Treatment 3
Cow manure 1kg	Cow manure 1kg	Cow manure 1kg	Cow manure 1kg
Leaves 1 kg	Leaves 1 kg	Leaves 1 kg	Leaves 1 kg
No *Bacillus* sp. added	200 mL of 6.0×10^{10} cfu mL^{-1} *Bacillus* sp. added	200 mL of 6.0×10^{10} cfu mL^{-1} *Bacillus* sp. added	200 mL of 6.0×10^{14} cfu mL^{-1} *Bacillus* sp. added

Composting Setup and Experiment

An apparatus was designed where composting treatments were kept in glass containers. These containers were kept in a tank containing water and temperature was maintained at a range of 30-35°C (Fig. 2) by heating water with an electric rod. Composting was performed for 60 days and samples were taken for 0, 15, 30, 45 and 60 days. Moisture content was maintained at 50-55% with frequent checking and testing. According to Gajalakshmi and Abbasi (2008), the ideal moisture content for an efficient composting lies between 50-60%, which is ideal for microbial metabolism. Carbon content, pH, nitrogen concentration, phosphorus concentration, C/N ratio, temperature and *E. coli* colony formed units were measured.

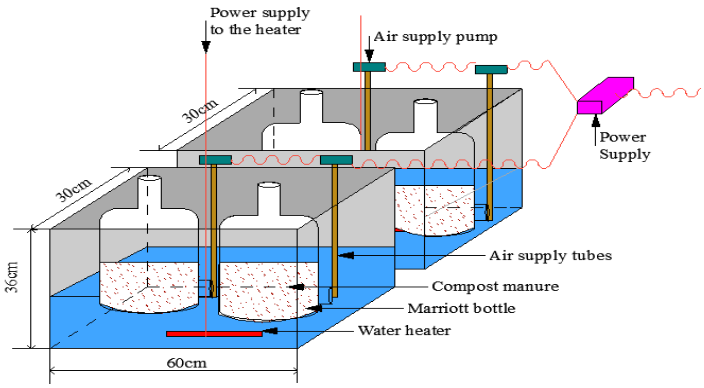

Fig. 2 Apparatus designed for composting

RESULTS AND DISCUSSION

Evaluating Fermentation with *Bacillus* sp.

The result of temperature (Fig. 3) shows that there is difference in highest temperature obtained compared with control and *Bacillus* sp. added treatments. The highest temperature obtained by Control, Treatment 1, Treatment 2 and Treatment 3 were 55 °C, 59 °C, 58 °C, 60 °C respectively. The high temperature can be corresponded to heat generated by microbial activity of added *Bacillus* sp. compared to control. The temperature did not reach a very high level (above 65 °C), which is considered harmful for microbial growth (Valente et al., 2009). Also, very low temperature is unfavorable for composting as it delays decomposition (Bernal, Alburquerque and Moral, 2009).

Maintaining an appropriate pH in a compost pile is vital for the survival of beneficial microbes. The pH of all the treatments was in between the range of 6.8 to 7.5 (Fig. 4), which indicated that a favorable condition existed for growth and metabolism of microorganisms.

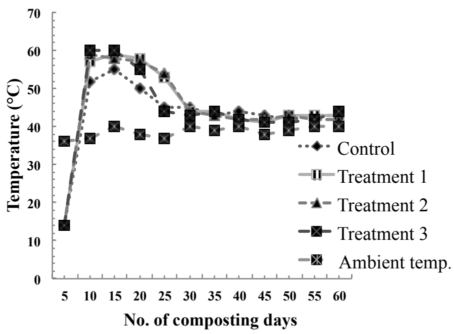

Fig. 3 Changes in temperature with time

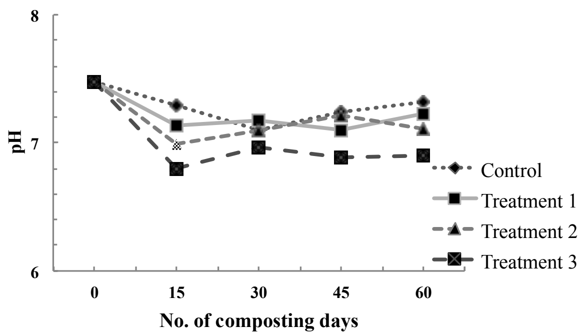

Fig.4 Changes of pH with time

Table 2 shows the total numbers of colonies formed of *E. coli* for different treatments. After 15 days of composting, there were no colonies formed with *Bacillus* sp. added treatments. This can be attributed to the heat generated during metabolism and competition of nutrients by microbes. According to Haug et al., (1980) pathogenic microbes die if there is temperature above 55°C for 3 or more days. On the other hand, there were 5.1× 10^3cfu g^{-1} of *E. coli* colonies in control during the first 15 days. At 30 days of sampling colonies of *E. coli* were not found in control as well.

Table 2 *E. coli* (cfu g^{-1}) in different treatments

	0 days	15 days	30 days	45 days	60 days
Control	9.3 × 10^3	5.1× 10^3	0	0	0
Treatment 1	9.3 × 10^3	0	0	0	0
Treatment 2	9.3 × 10^3	0	0	0	0
Treatment 3	9.3 × 10^3	0	0	0	0

Of the many elements required for microbial decomposition, carbon and nitrogen are the most important. Carbon provides both an energy source and the basic building block making up about 50 percent of the mass of microbial cells. As seen in Fig. 5 the carbon percent decreases with increase in composting days. After 60 days, carbon percent of Control was 40.2, Treatment 1 was 38.45, 2 Treatment 2 was 38.45 and Treatment 3 was 36.18 percent. This result can be explained as number of colonies of added *Bacillus* sp. increased there was higher decomposition of organic carbon. Similarly C/N ratio (Fig. 6) observed, had significant difference in decrease between control and *Bacillus* sp. added treatments.

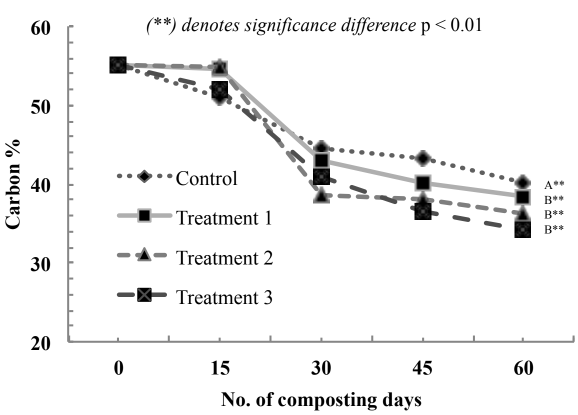

Fig. 5 Decrease in carbon percent with time

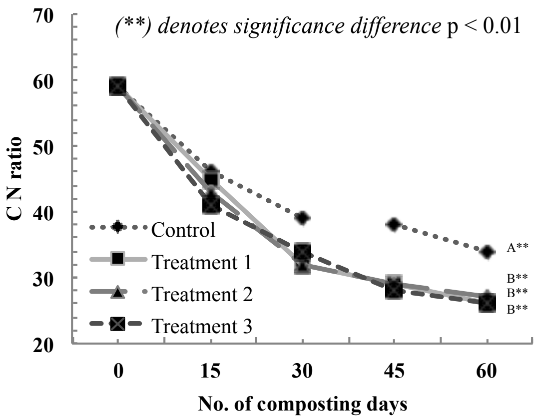

Fig. 6 Decrease in C/N ratio with time

According to Tumihairwe et al., (2009) large organic loss suggests pronounced microbial activity. This supports the obtained result where added *Bacillus* sp. had resulted in increased decomposition rate of carbon decreasing the carbon percentage and C/N ratio.

The nitrogen (Fig.7) and phosphorus concentration (Fig.8) of all the treatments showed increase with increase in composting days. The final concentration of total nitrogen at 60 days for control, treatment 1, treatment 2 and treatment 3 were 6825.07, 7728.02, 7835.02 and 8457.71 mg^{-kg} respectively. According to Viel et al., (2007) increase in total nitrogen value might increase due to nitrogen fixing bacteria that commonly occurs at the end of composting. The results obtained contradict to Tognetti et al., (2007) who showed that total nitrogen decreased during composting. Decrease in nitrogen content may occur due to leaching and volatilization, but this experiment was conducted in a closed container nullifying the effects of leaching.

Likewise, After 60 days of composting the total concentration of phosphorus for Control, Treatment 1, Treatment 2, and Treatment 3 were 2728.1, 3178.29, 3168.52, 2920.52 mg kg^{-1}

respectively. The result obtained matches with those of Felton et al., (2004) and Chandna et al., (2013) where they found increase in total phosphorus.

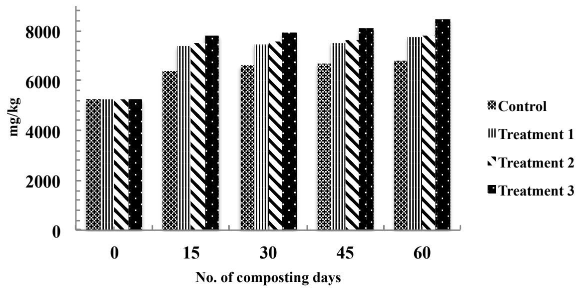

Fig. 7 Change of nitrogen concentration with time

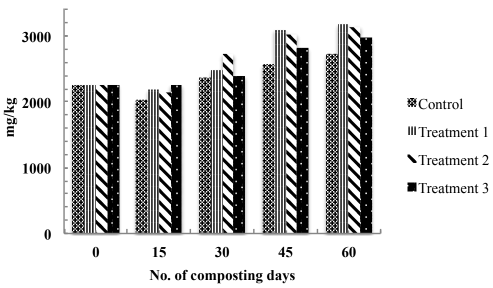

Fig. 8 Change of phosphorus concentration with time

Plant Growth Experiment

A plant growth experiment was conducted to analyze the effects on growth of plants using the compost that was made. The plant used was *Brassica rapa* (Komatsuna). Twelve pots were used; three each for Control, Treatment 1, Treatment 2 and Treatment 3 (unit area is 1/10000 a, and a height of 150 mm). Six hundred grams of soil was used in each pot with 350 grams of compost applied. 100 ml of water was put in the pots after three days interval. Environmental conditions like temperature, light were made constant for all the pots. The height of the plants was measured after 3 days. The experiment was conducted for the period of 30 days from July 2017 to August 2017.

Table 3 Physical and chemical properties of soil used

Water content (%)	10.7±0.10
Organic content (%)	14.87±0.17
EC (mS/cm)	1.33±0.03
pH	5.22±0.04
Total nitrogen Mg Kg^{-1}	1590.36±0.72
Total phosphorus Mg Kg^{-1}	907.65±0.03

Note: Values are mean ± SD (n=3).

In the result (Fig. 9) of plant growth experiment, the treatments with added *Bacillus* sp. had higher crop height compared to control with significant difference at 99%. There was no significant difference in height of plants with *Bacillus* sp. added treatments. The heights of plants for Treatment 1, Treatment 2 and Treatment 3 were 14.97, 14.56 and 14.3 cm respectively. With the obtained results it can be said that plants grown in compost made with added *Bacillus* sp. can help in growth of plants which coincides with results obtained by Toyota and Watanabe (2013) who found that *Bacillus* sp. helps in development of plant by suppressing disease and enhancing plant growth.

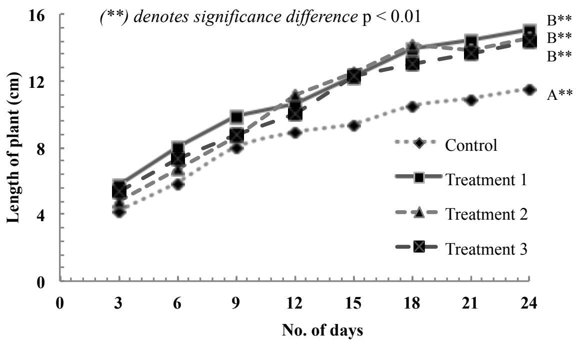

Fig. 9 Increase in plant growth with time

Fig. 10 Plant grown for two treatments

CONCLUSION

This research was conducted to examine if adding of *Bacillus* sp. is beneficial for enhancing fermentation, better decomposition of farmyard manure and also elimination of pathogenic microbes from it. The results showed that adding of *Bacillus* sp. was effective in increasing the temperature, thus eliminating of pathogenic microbes. Additionally, increased microbial activity helped in better decomposition of carbon matter and better C/N ratio. Further, there was increase in nitrogen and phosphorus concentration in *Bacillus* sp. added treatments compared to control. The plant growth experiment proved that compost prepared with added *Bacillus* sp. can help in development of plants.

Thus, with the observed results we can conclude that adding *Bacillus* sp. is beneficial for better farmyard manure. Further research is required to identify specific species of microorganisms involved during composting. Also, field experiments should be conducted to determine the loss of nutrients through leaching, runoff and volatilization.

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