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Studies on Bio-composting of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulasi) leaves

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ABSTRACT

In developing countries like India, rapid population growth, urbanization, and industrialization have led to a significant rise in solid waste generation, posing challenges for municipal authorities. Improper disposal of leaf litter exacerbates the problem. To tackle this issue, the present study focused on eco-friendly and cost-effective bio-composting of leaf litter using traditional enhancers such as animal waste and earthworms. The study involved preparing six composting piles with combinations of Neem leaf litter, Tulasi leaf litter, red soil, cow dung, buffalo dung, and goat manure in a 2:2:1 ratio. These piles were placed in bags in a dark room, and moisture content was maintained by adding water. The composting process included anaerobic composting for 48-72 hours followed by aerobic composting for 60 days. Throughout the process, various Physico-chemical, enzymatic, and microbiological parameters were analysed, including soil enzyme activities (such as amylase, cellulase, pectinase, xylanase, protease) and plant growth-promoting rhizobacteria (PGPR) activities (such as siderophore production, indole-3-acetic acid (IAA) production, and phosphate solubilization). Additionally, the impact of the compost on fenugreek plant growth was assessed. The results demonstrated significant changes in the compost's Physico-chemical and enzymatic parameters during the incubation period. Enzyme activities increased with the addition of animal dung. All six combinations of composting piles exhibited considerable improvements in shoot and root lengths, indicating successful plant growth. Notably, the combinations involving red soil, Neem leaf litter, and either buffalo dung or cow dung showed particularly favourable results. In conclusion, the study revealed the effectiveness of combining leaf litter and animal waste for composting, providing a successful and environmentally friendly approach to managing leaf litter.

Keywords: Leaf litter, Animal waste, Compost, Soil enzymes, PGPR

Introduction

Urbanization has led to a significant increase in the generation of solid waste, posing a major challenge for municipal authorities in cities. One particular issue is the accumulation of large

quantities of leaf litter in various areas such as forests, roadsides, playgrounds, and lawns (Singh *et al.,* 2017). This has been identified as a significant problem in India, where the prevailing practice involves piling up the leaf litter and setting it on fire. Unfortunately, this approach leads to the loss of valuable nutrients, including nitrogen, phosphorus, and organic carbon, as they are released into the atmosphere as ash. This contributes to air pollution and only minimal amounts of these nutrients are eventually retained in the soil (Hamzaoui- Azaza *et al.,* 2012).

Leaf litter indeed plays a crucial role in soil protection and enrichment. It serves as a protective layer, shielding the soil from direct exposure to solar heat and reducing the risk of soil erosion caused by wind and water. Additionally, leaf litter serves as a valuable food source for microbial communities residing in the soil. These microorganisms release enzymes that facilitate the decomposition of the organic matter present in the leaf litter. As a result, the nutrients contained within the litter become available for nutrient cycling in the soil, ultimately promoting plant growth (Abbasi and Ramasamy, 2001).

Furthermore, the activities of soil enzymes are essential for the stabilization of soil structure. Enzymes participate in various biochemical processes that contribute to the formation and maintenance of stable soil aggregates. These aggregates improve soil porosity, water infiltration, and nutrient availability. By enhancing soil structure, enzymes play a vital role in supporting healthy plant growth and overall soil health (Burns, 1983).

Composting has emerged as an appealing and integral approach in modern solid waste management practices. It offers an economically viable solution for handling organic solid waste. The end product of composting, which is derived from the decomposition of organic waste materials, serves as a valuable source of high-quality nutrients and can be effectively utilized as a nutrient-rich manure for agricultural and horticultural purposes (Sharholy *et al*.,2008).

Composting not only provides a means of waste management but also contributes to environmental sustainability. Organic fertilizers produced through composting are eco-friendly and do not pose harm to natural resources. Instead, they contribute to improving soil fertility, leading to healthier plant growth. By incorporating organic fertilizers into the soil, the levels of soil organic matter are increased, promoting soil structure and water-holding capacity. Moreover, the use of organic fertilizers enhances soil microbial biomass and activities, creating a favorable environment for beneficial soil microorganisms that support nutrient cycling and overall soil health (Acosta-Martinez and Tabatabai, 2000). Therefore, composting presents a sustainable and environmentally friendly solution for solid waste management, allowing the conversion of organic waste into a valuable resource that enhances agricultural productivity and minimizes reliance on synthetic fertilizers.

Growth and survival of microorganisms are important in agricultural soils that depend on the carbon source contained in the cellulose occurring in the soils whether in the form

agricultural waste or cattle feed waste (Deng and Tabatabai, 1994). For example, the enzyme cellulase (EC 3.2.1.4) is a complex enzyme which plays an important role in bioconversion of cellulose to simple soluble reducing sugars. The phosphatase (EC 3.1.3.1) is an extracellular enzyme which hydrolyses the organic phosphates to inorganic phosphate and plays an important role between biologically unavailable phosphorus and available phosphorus (Kunc, 1979). It plays a key role in the soil ecosystem and it is a good indicator of soil fertility by including other enzymes like amylase (EC 3.2.1.1), pectinase (EC 3.2.1.15) and xylanase (EC 3.2.1.8) (Dick and Tabatabai, 1992).

Compost	Material (2:2:1)

An attempt was made in this study to observe the influence of leaf litter and animal waste as an additive on soil physico-chemical, biological and enzyme properties during composting (Narasimha and Sridevi, 2013). Previously a study was performed by Jesikha *et al* (2012) to evaluate the potential of epigeic earthworm *Eudrilus eugeniae* to convert waste leaf litter and waste cattle manure into vermicompost in 90 d, Jayanthi *et al* (2010) processed mixed leaves litter in which the litter was mixed with cured cow dung in different proportions and composting of municipal solid waste, leaves and poultry waste was done by Yaghmaeian *et al* (2005) by pit and wind row process. Therefore, our approach is a bit more innovative by using a simple compost pile method prepared in used plastic urea bags and producing compost in less time (60 d) from leaf litter and animal waste.

Materials and Methods

Collection of samples

Compost piles were prepared in polyhouse during November month at the Bhavan's Vivekananda college campus, Sainikpuri, used urea bags, using leaf litter of *Azadirachta indica* and *Ocimum sanctum* collected from college campus, and local temples respectively. Buffalo, cow and goat dung were collected from local dairy farms. Chopping of leaf litter was done to bring down the particle size to 2-5 cm.

Preparation of compost piles

The present study was conducted to monitor changes in the Physico-chemical parameters by preparing six different combinations for 60 d. Compost piles were prepared using different combinations of red soil, leaf litter (*Azadirachta indica* and *Ocimum sanctum*) and animal waste i.e., cow dung, buffalo dung, goat manure dung separately in the ratio of 2:2:1(Table 1) along with a control.

Control	Only red soil
Combination 1	Red soil+ Neem leaf litter+ Buffalo dung
Combination 2	Red soil +Neem leaf litter+ Cow dung
Combination 3	Red soil +Neem leaf litter + Goat manure
Combination 4	Red soil +Tulasi leaf litter+ Buffalo dung
Combination 5	Red soil +Tulasi leaf litter+ Cow dung
Combination 6	Red soil +Tulasi leaf litter + Goat manure

Table 1: Different combinations of the compost in the ratio of 2:2:1

Bags were tied and placed in a dark room. Water was added to maintain the moisture content (50–60%). Anaerobic composting was carried out for 48-72 hr and later aerobic composting was done for 60 d. For every 5d composting soil sample Physico-chemical properties like temperature, odor, soil texture, humidity, pH and color were monitored using standard methods (APHA- 2000).

Physico-chemical studies

Measurement of temperature

The temperature was measured periodically (5 days' interval) using mercury thermometer by placing 10 cm deep into the compost piles.

Measurement of moisture content

About 5 g of soil sample was taken from composting piles and kept in a hot air oven for 24 hr. After 24 hr the weight of the soil was measured. The difference between the initial and final weight gave the moisture content of soil (Godishala *et al*., 2019).

Enumeration of microorganisms

After 60 d of incubation, compost soil samples were analyzed for different micro flora by using serial dilution method, where diluted samples were inoculated onto different agar plates like Nutrient agar (NA), Yeast extract potato dextrose agar (YEPD) and Potato dextrose agar (PDA) respectively and incubated for required time periods accordingly. Obtained microbial colonies were numbered and colony morphology was studied along with staining reactions using Gram's staining for bacteria and yeast, Lactophenol cotton blue staining for fungal colonies. Subsequently, colonies were sub-cultured on agar slants for further studies (Ryckeboer *et al.,* 2003).

Degradative efficiency of compost isolates

Primary screening was conducted on starch agar medium (Alariya *et al.,* 2013), cellulose agar medium, casein agar medium, xylan agar medium and pectin agar medium (Chaitanya *et al.,* 2016; Singh *et al.,* 2015) for amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4), protease (EC 3.4.21.112), xylanase (EC 3.2.1.8) and pectinase (EC 3.2.1.15) producers respectively. The positive strains, which exhibited clearance zones around the colony after incubation, were isolated and maintained on agar slant at 4 °C for secondary screening in submerged fermentation (liquid medium) (Nawawi *et al*., 2017).

Following primary screening, the bacterial cultures were further narrowed down by secondary screening in a submerged fermentation medium. In secondary screening, the isolates were carried out in 250 mL Erlenmeyer flasks containing 50 mL of basal medium. The basal medium was supplemented with 1% starch, 1% carboxymethyl cellulose, 1% sodium caseinate, 1% beechwood xylan and 1% pectin separately for amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4), proteinase (EC 3.4.21.112), xylanase (EC 3.2.1.8) and pectinase (EC 3.2.1.15) assay respectively and incubated at ambient temperature for required time period. The cell supernatant was used for enzyme assay along with a blank (Dhaver *et al*., 2022).

PGPR studies
Production of Indole acetic acid

Indole acetic acid production was studied in accordance with Bric *et al* (1991) where different cultures supernatant was mixed with few drops of ortho phosphoric acid followed by Salkowski's reagent (35 % perchloric acid and 0.5M $FeCl_3$ solution). Development of pink color indicates IAA (Indole acetic acid) production.

Siderophore production

Siderophore production was detected by adding 0.5 M ferric chloride solution on to the inoculated agar plates respectively and observed for color change (Schwyn and Neilands, 1987).

Phosphate solubilization

The isolates obtained were aseptically inoculated onto Pikovskaya's agar plates and after incubation for an appropriate time, colonies showing clear zones were considered as positive (Vassilev, and Vassileva 2003).

Plant growth studies
Seeds of fenugreek plants were obtained from the Govt. District Agriculture institute, Hyderabad. The study of the effects of isolated strains on plant growth was carried out in pots using all combinations of compost soil formed by leaf litter and red soil as a control.

Shoot and root length
The seedlings of *fenugreek* were plucked out from the pots carefully and washed with tap water to remove the adhering soil. Shoot and root length were measured from the base to the tip of the lengthiest shoot and root respectively with the help of a ruler.

Results and Discussion

In this study, small composting piles were created using six urea bags measuring 20 x 12 cm (Fig. 1). The composting piles consisted of different combinations of red soil, leaf litter from Azadirachta indica and Ocimum sanctum, and three types of animal waste (cow, buffalo, and goat dung) in a ratio of 2:2:1, as indicated in table 1. A control group consisting solely of red soil was also included.

This approach differs from previous studies in several ways. Mushan et al (2012) focused on the vermicomposting potential of the earthworm *Eudrilus eugeniae* for converting waste leaf litter and cattle manure into vermicompost over a 90-day period. Vasanthi et al (2013) explored the composting process by mixing leaf litter with cured cow dung in varying proportions. Munnoli et al (2009) conducted composting of municipal solid waste, leaves, and poultry waste using pit and windrow methods.

In contrast, the current study introduces a unique element by utilizing small composting piles created in urea bags. The combination of red soil, leaf litter, and different types of animal waste in specific ratios offers a novel approach to composting. By including a control group consisting solely of red soil, the researchers can compare the effects of the additives on the physico-chemical, biological, and enzyme properties of the soil during the composting process.

Fig. 1: Compost piles prepared using waste urea bags

Physico-chemical properties of soil

Analysis of compost soil samples revealed that leaf litter underwent changes in all measured parameters. In the first few days' anaerobic conditions were maintained during composting so that pathogenic organisms get killed which were present in composting piles, which was observed in the form of gasses production with unpleasant smell may be due to the production of methane and hydrogen sulfides. Later aerobic conditions were maintained and the composting speeds up and the unpleasant smell gradually decreases. After 60 d there was almost a decrease in the unpleasant odor from the composting bags.

In the initial days of composting, the soil was hard and the soil color was the same. Later from the 30th day the soil texture started to become soft and there was a small change in the color, indicating degradation by microorganisms. After 60 d, the soil was almost soft and turned into black color.

First few days of the composting process, the temperature was low and rise in temperature of about 31 ^{0}C, indicated that degradative organisms were able to degrade the waste. Later there

was a decrease in temperature and maintained a constant temperature level of 25 ^{0}C, indicating the maturation phase in almost all combinations of compost (Fig. 2).

Moisture content was maintained in the composting bags by sprinkling water. At the end of the composting, humidity was checked (Table 2) and was found to be high in the combination 2 (Neem: cow dung) compost soil.

Fig. 2: Measurement of temperature during composting for about 60 days

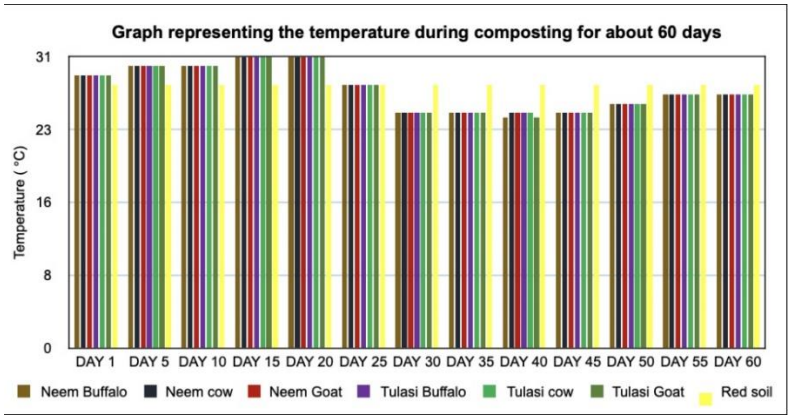


Table 2: Percentage of the Humidity of Soil samples

S.No.	Sample	Humidity %
1	Red soil(control)	50.00%
2	Combination 1	50.01%
3	Combination 2	60.07%
4	Combination 3	55.30%
5	Combination 4	57.59%
6	Combination 5	52.00%
7	Combination 6	55.07%

These improvements in higher organic content in compost soil may be due to the long-term deposition of organic waste in the form of leaf litter and animal waste (table 3). Similar results were reported by Zande (1996), Dodor and Tabatabai (2003), Nizammuddin *et al* (2008) due to the discharge of dairy effluents, increase of organic matter with enhanced soil enzyme activities.

Table 3: Physico-chemical properties of compost soil

Properties	Before composting	After composting
Color	Brownish red	Black
Odor	Pleasant	Unpleasant

Humidity (%)	50	60.07
Temperature (^{0}C)	20	31
pH	Neutral	Slightly alkaline
Organic matter (kg/g)	4.0	6.1

The different combinations of compost soil samples were inoculated on to different agar plates like Nutrient agar (NA), Yeast extract potato dextrose agar (YEPD) and Potato dextrose agar (PDA) respectively and about 58 isolates were studied microscopically (Fig. 3) by using respective staining techniques and percentage of different isolates were shown in the form of a pie chart (Fig. 4).The highest percentage of isolates were belonged to Gram positive bacilli (21 %) followed by fungal- *Aspergillus spp*. (19 %) and yeast about 2 %.

Fig. 3: Few microscopic observations of compost isolates

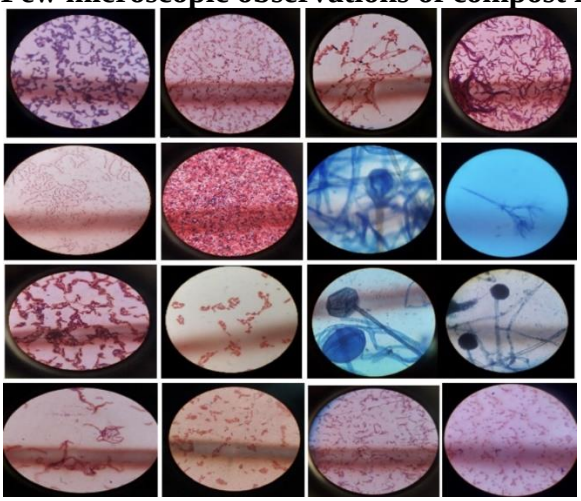
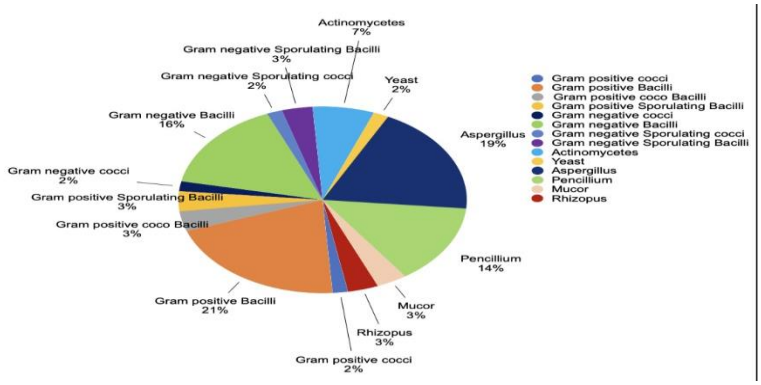
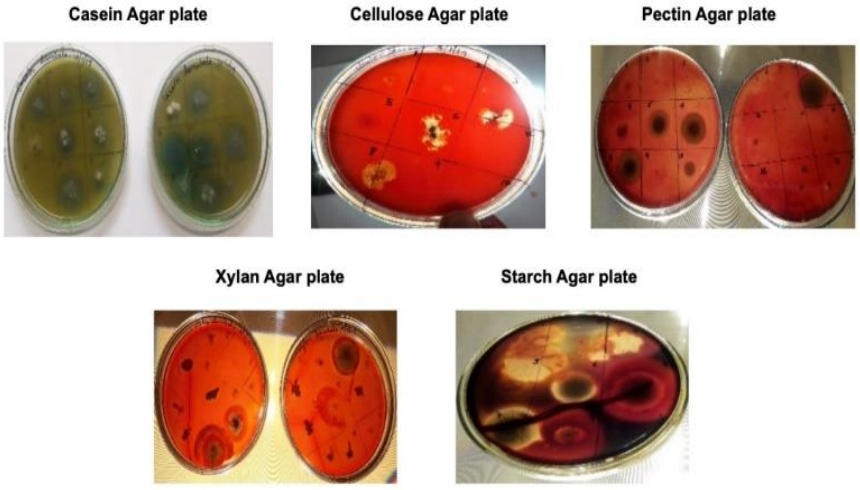


Fig. 4: Percentage of different compost isolates



The successful composting requires a level of interaction among microorganisms as the degradation of organic matter present in leaf litter relies on the activity of various enzymes like amylase, cellulase, pectinase, protease and xylanase. As a result, in present work degradative studies resulted in 32 isolates, which displayed the zone of clearance on different agar media. Therefore, the growing colony resulted in the clear zone formation and it was possibly because of the regional presence of enzymes and its degradation by respective isolates. Out of 32 isolates, 13 isolates showed positive results for amylase production, 20 isolates for xylanase production, 3 isolates for cellulase production, 4 isolates for pectinase production and 19 isolates for protease production. This clearly indicated that organisms were able to degrade the organic matter in the compost (Fig. 5).

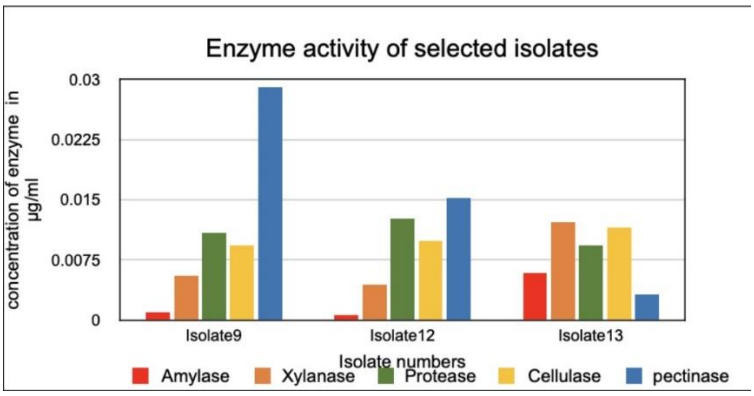
Fig. 5: Plate assay methods for various enzymes



However, few isolates (numbered as 9, 12 and 13) showed the concurrent reaction of zone of clearance in all the agar medium tested. Therefore, these three multi enzyme producers were selected for secondary screening, in order to determine the quantitative analysis of the enzymes produced. The enzyme activities of these three isolates were recorded (Fig. 6). For isolates 9,12 and 13 the amylase activity was 0.000947µg/mL, 0.000654 µg/mL, 0.00591 µg/mL, xylanase activity as 0.0054 µg/mL, 0.0043 µg/mL, 0.012 µg/mL, protease activity as 0.0108 µg/mL, 0.0126 µg/mL, 0.0092 µg/mL, cellulase activity as 0.0093 µg/mL, 0.0098 µg/mL, 0.0115 µg/mL and pectinase activity as 0.028 µg/mL,0.015 µg/mL and 0.0032 µg/mL respectively.

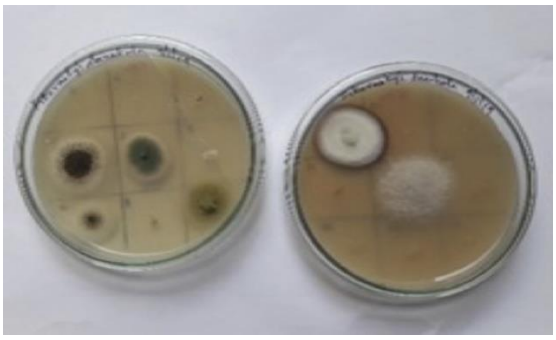
The higher multi enzyme activities in the present study would be attributed to the presence of higher organic content of leaf litter used and diversified microbial population due to addition of additives like animal waste to the red soil during composting. According to Nandana *et al.*, 2012, and Srilakshmi *et al., (*2012), discharge of effluents from cotton ginning mill and litter soil improved the enzyme activities.

Fig. 6: Graph showing the enzyme activity of selected isolates



PGPR effects of compost were studied because IAA is the general product of L- tryptophan metabolism produced by microbes. IAA is the common auxin hormone of plants that helps for the plant growth. About 21 isolates were positive, 7 isolates showed partial positive and 4 showed negative. Isolate 8 has shown a strong positive result for IAA producers. Iron is recognized as an essential micronutrient for the plant growth, it was found to be abundant in the soil but plants can utilize Fe^{+2}, siderophore producer are Iron chelators that helps in conversion of Fe^{+3} to Fe^{+2}, so that plant can utilize $Fe^{+2.}$ About 23 isolates showed positive results, 2 isolates showed partial positive and 7 shown negative. Isolate 9 and isolate 23 has shown a strong positive result for siderophore. Phosphate solubilizers are the microbes that help in solubilizing the inorganic phosphorus to insoluble compounds, so that plants can utilize the phosphorus which is one of the major essential macronutrients for plants. Positive results were obtained as a zone of hydrolysis (Fig. 7). Nine isolates showed positive results and isolate 53 has shown a larger diameter of about 1.1 cm as shown in figure 7. Therefore, the results clearly indicate that isolate 8 and 36 can be considered as multi enzyme producers.

Fig. 7: Phosphate solubilizers

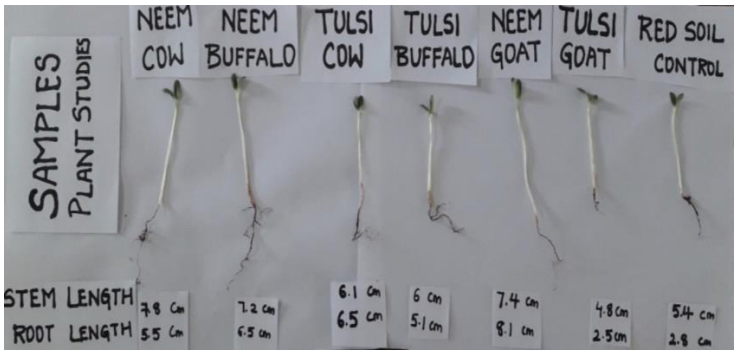


Further, Fenugreek plant growth studies were done by observing the effect of six different combinations of nutrient rich compost soil along with the red soil as control. All the six samples showed a faster growth rate of fenugreek plants when compared to control. Combinations 1,2, 4 and 5 had supported very good growth and remained fresh for several days, but the plants grown with combination 3 and 6 had spoiled rapidly within 2-3 days of the plant growth (Fig. 8).

Fig. 8: Growth of fenugreek plants in different combinations of compost

All the combinations showed a significant increase of their efficiency on the growth of shoot and roots. The best results were achieved by the combination of compost using [Red soil: Neem leaf litter: Buffalo dung] and [Red soil: Neem leaf litter: Cow dung] as 7.2 cm (shoot), 6.5 cm (root) and 7.8 cm (shoot),5.5 cm (root) when compared with control (only red soil) as 5.4 cm (shoot) and 2.8 cm(root) respectively (Fig. 9). Similar observations were made for the black gram plants by Prakash *et al.*, 2013 clearly indicated the production of healthy seedlings.

Fig. 9: Measurements of stem and root length of fenugreek seedlings



Conclusion

Leaf litter is a good source of organic waste. The degradation of leaf litter can be done efficiently by using animal waste as an additive. Managing leaf litter by composting is an eco-friendly and alternative approach to waste management since the leaf litter will neither be burnt nor be landfilled. This will also help in reduction of air pollution produced by burning of leaf litter. Leaf litter compost can be further re-used as fertilizers as it is rich in nutrients, carbon and nitrogen sources, multi enzyme production capabilities help in degrading the complex organic substances and good PGPR potentials. All these properties will be helpful in organic farming which results in increased productivity of crops. If neem and tulasi leaf composting retains limonoids, may help to control soil plant pathogens and eugenol and triglyceride may control pests, thereby farmers would be more benefited. Ultimately leaf litter utilization in composting will help in making the residential areas cleaner.

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