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Sustainable Management Technique for Recalcitrant Leaf Litter of *Mesua ferrea* L. in Avenue Plantations

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Abstract

Avenue plantation produces a significant amount of leaf litter throughout the year, and sustainable management is a primary concern in various establishments. Mesua ferrea L. (MF) is a widely grown avenue tree species that produces enormous amounts of leaf litter throughout the year. The litter remains undecomposed in natural conditions for a long time (2-3 years), requiring labour-intensive management. The rate of decomposition, nutrient dynamics and microbial species involved in the decomposition process has been studied by the modified litterbag method in natural conditions using intact and shredded MF leaf litter. The results of the study revealed 50% biomass loss from intact and shredded MF leaf litter within 120 days as compared to the negligible biomass loss in the control treatment. The percentage of nutrients released followed the same pattern as biomass loss for intact and shredded MF samples. The decay constant values were observed as 1.99, 1.92 and 0.33 for shredded, intact and control treatments, respectively. Penicillium with 12 isolates was recorded as the most dominant cellulose-degrading fungi during the decomposition of MF leaf litter. The study observed that the embedding of shredded MF leaf litter in soil significantly reduced the time required for the decomposition of litter. It is concluded that embedding shredded MF leaf litter in the soil can enhance rapid decomposition while improving soil fertility. The proposed technique may be employed in the sustainable management of MF leaf litter in particular and other litter in general.

Introduction

Planting trees along the roadsides of highways and pathways is known as avenue plantation.¹⁻² It is

practised for enhancing aesthetic value of premises and highways, to provide shade, protect soil erosion, and production of timber, flowers and fruits.

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Keywords Avenue Plantation; Decomposition; Fungi; Leaf Litter; *Mesua ferrea* L. The plantations also provide a sink for carbon dioxide,³⁻⁵ release fresh oxygen⁶ and improve soil fertility. Avenue trees are commonly planted along roadsides in institutional campuses, commercial complexes, government establishment premises and residential areas, according to National Forest Policy (1952). A few of the most preferred tree species used for avenue plantations include Saraca indica (Ashoka tree), Delonix regia (Gulmohar), Messua ferrea L. (Nahar), Mimusops elengi (Maulsari/ Bakul), Anthocephalus cadamba (Kadam), Elaeocarpus granitus (Rudraksha), Terminalia chebula (Arjuna) and Gravillea robusta.7 Generally, perennial and evergreen tree species are selected for avenue plantations since they produce green leaves throughout the year and possess an ornamental tree canopy and colourful flowers.

Although avenue plantations provide multiple benefits, they also pose a major management problem due to the accumulation of a significant amount of leaf litter.⁸ The most widely adopted management practice for leaf litter in avenue plantations involves either disposal through municipal waste management systems or burning in the corner of the premises or at the collection site along plantation areas, which is not a standard protocol prescribed under Solid Waste Management Rules (2016). It is well known that the burning of leaf litter causes the immediate release of various gases such as CO₂, CO, SO₂ and other airborne particulate matters in the atmosphere, thereby enhancing global warming and air pollution.⁹⁻¹⁰

The natural decomposition of leaf litter from certain tree species such as Messua ferrea L., Ailanthus grandis, Castanopsis indica, and Vatica lancefolia has been reported to be slower as compared to other leaf litter due to the presence of higher content of lignin, lower nitrogen contents¹¹⁻¹⁴ and presence of phenolic compounds in the leaf tissues.¹⁵ The major problem of MF leaf litter is recalcitrance, leading to very slow natural decomposition with an average life span of three years. Naturally, microorganisms decompose leaf litter under suitable environmental conditions¹⁶ and obtain nutrients such as C, N, P, Ca, Mg, etc. They play major roles in nutrient recycling by releasing these nutrients back into the soil¹⁷⁻²¹ through enzyme-catalyzed reactions during the process of plant litter decomposition.22-24 Among microorganisms, bacteria and fungi are known to decompose cellulose,²⁵ while fungi are the main decomposers of cellulosic matter by releasing cellulase enzymes.²⁶⁻²⁷

The need to recycle organic wastes to enhance soil fertility has increased in recent years due to the high cost of fertilizers and decreased availability of organic manures.²⁸⁻²⁹ Despite widespread avenue plantations inside the institutional campuses, premises of government establishments and commercial complexes, no sustainable leaf litter management practices have been developed with reference to a specific plant species. Therefore, the present study aimed to evaluate the decomposition rate of MF leaf litter in natural conditions and evaluate nutrient dynamics for developing an effective and sustainable management strategy.

Material and Methods

Study Site

The present study was conducted in the avenue plantation inside the campus of North Regional Institute of Science and Technology (NERIST), Deemed-to-be-University, Nirjuli, Arunachal Pradesh. The campus avenue plantation is dominated by more than 160 evergreen trees of *Mesua ferrea* L. (MF) trees, commonly known as Nahar or Nageshwar (Hindi), along the roadsides, covering an area of 2.34 hectares within the campus. The MF tree has a beautiful canopy and remains covered with green foliage throughout the year, producing considerable leaf litter (solid biomass).

Collection of Leaf Litter and Preparation of Litterbags

Freshly fallen MF leaf litter was collected by constructing a green net trap under the tree canopies to avoid exposure to the soil surface. The MF leaf litter was oven-dried at 60° for 48h. The oven-dried MF leaf litter was divided into two parts: intact (without shredding the leaves) and shredded (by breaking down into 2-3 cm-sized fragments). For the control treatment, intact MF leaf litter was used. The litterbags of uniform size (20 cm x 20 cm) to hold 10g of MF leaf litter were prepared using a nylon net having a mesh size of 2 mm.

Decomposition of MF leaf Litter

The decomposition rate and nutrient dynamics of the MF leaf litter were studied in natural conditions using the modified litterbag method.³ A total of six (6) pits

of 1 ft² were dug out by keeping a minimum distance of 1 metre apart between 2 pits within the institute campus. The pits were embedded with 6 litterbags (3 each intact and shredded MF leaf litter). Thus, a total of 36 (6 x 6) litterbag samples were prepared to be embedded in the soil. After embedding in the pits, the litterbags were covered with a layer of soil, marked by a labelled peg, to enable identification of the exact location of the litterbags during sample collection. Close to the vicinity of the embedded litterbags, three control plots, each 1m² were prepared by spreading the intact leaves (10 g) on the ground surface of the soil to enable a natural decomposition process without embedding in the soil. The sample plots were well protected by erecting agro-net fences and roofs. The litterbag samples and control samples were studied for a year (360 days) and periodically removed for analysis.

Sample Collection and Analysis

The litterbag samples were recovered from each pit after 60, 120, 180, 240, 300 and 360 days, respectively. The recovered litterbag samples were carefully put in sterile polythene bags and brought to the laboratory for analysis. Subsamples weighing 5 gm were taken from the litterbags and stored in a refrigerator at a temperature of 4° C for microbial analysis. Unlike the litterbag samples, the control samples were left undisturbed and recovered at the end of 360 days.

Determination of Biomass Loss

The recovered litterbags from the pits were washed in a plastic bucket or tub with tap water by swirling briefly and carefully decanting through a 2 mm mesh size sieve to remove extraneous matter such as soil, roots and sediments. Such brief washing allows the leaching of nutrients.³¹ Then, cleaned MF leaf litter samples were drained off to remove excess water and oven-dried at 60°C for 48 hours, and then the dry weight was recorded. An attempt was also made to find out the statistical significance of the biomass loss with respect to time using a simple linear regression equation (Y=a+bx).³²

Nutrient Content in MF Leaf Litter

The oven-dried MF leaf samples were grounded to a fine powder (60 mesh sieve < 0.25 mm). The nutrient contents of the MF leaf litter sample (250 mg) were analyzed from the aliquots obtained from the acid digestion of the samples according to the standard methods.³³⁻³⁴ The total nitrogen (TN) was determined using a micro-Kjeldahl distillation method and total phosphorus (TP) using the colourimetric method with pH adjustment. The total organic carbon (TOC) content was obtained by hot digestion of the powdered MF leaf litter (50 mg) with concentrated sulphuric acid (H_2SO_4) and an aqueous potassium dichromate ($K_2Cr_2O_4$) solution, followed by titration with ferrous ammonium sulphate [(NH_4)₂Fe(SO_4)₂·6H₂O] solution.

Estimation of Annual Decay Constant (k)

Olson's (1963) decay model³⁵ has been employed to assess the differences in MF leaf litter decomposition rates for the control, intact and shredded leaf litter. According to this model decay constant (k) was calculated as follows:

 $\ln(X_{t}/X_{0}) = -kt$

 $k=-(ln(X_t/X_0)/t$

Where X_0 = Initial dry weight, X_t = Remaining dry weight after the time (t), t = Time (year), In = Natural log

The time required for the MF leaf litter to decompose, 50% ($t_{_{1/2}}$) and 99% ($t_{_{99}}$) of its dry weight, were calculated as follows;

 $t_{\frac{1}{2}} = 0.693/k$ $t_{\frac{99}{2}} = 5/k$

The decay constant (k) value directly corresponds to the decomposition rate, i.e., a higher k value implies a higher decomposition rate. In addition, a higher k value will have lesser $t_{1/2}$ and t_{99} , implying that the time required for decomposition will be less.

Determination of Nutrient Released

The nutrient released from the MF leaf litter during decomposition was calculated (%) as minus nutrient remaining (NR) from 100 per cent at the time of sampling.³⁶

NR (%)=C/Co*DM/DMo*100 Nutrient Released (%)=100-NR(%)

Where NR (%) = Nutrient Remaining

C = Concentration of element at the time of sampling,

Co = Initial Concentration of the element prior to decomposition,

DM = Dry mass at the time of sampling and

DMo = Initial dry mass of the sample prior to decomposition.

Screening of Cellulase Activity for Isolated Fungi Five-gram (5gm) subsamples from the litterbags were suspended in 95 ml sterilized distilled water, followed by serial dilution up to 10⁻³. A volume of 100 µl of inoculum from the 10⁻³ dilution was evenly spread on the solidified potato dextrose agar (PDA) media plates and incubated at 28°C for 4-7 days to isolate fungi. Pure fungal isolates were obtained by sub-culture of fungal isolates from mixed plates on fresh PDA plates and slants and stored at 4°C.

Cellulase activity was tested using Mendel and Reese Agar (MRA) media³⁷ supplemented with 0.5% Carboxymethyl cellulose Sodium salt (CMC-Na) containing KH₂PO₄ (2g), NH₄SO₄ (1.4g), MgSO₄.7H₂O (0.3g), CaCl₂ (0.3g), Yeast extract (0.4g), FeSO,.7H2O (0.005g), MnSO, (0.0016g), ZnCl₂ (0.0017g), CoCl₂ (0.002g), CMC-Na (5g), Agar (5g), distilled water (1000 ml) and final pH adjusted to 5±0.2. The purified fungal isolates were inoculated in the petri dish containing 20 ml of MRA CMC-Na media and incubated at 28°C for 4-7 days until the growth reaches a minimum diameter of 1-2 cm. After attaining the desired growth, the plates were flooded with 10 ml of 1% Congo red solution for staining³⁸ and were allowed to stand for a minimum of 15 minutes. The excess Congo-red stain was drained off, and the plates were de-stained by flooding with 1M Sodium chloride (NaCl) solution for 30 minutes. After destaining, NaCl was discarded, and the plates were analyzed for the formation of the hydrolyzing zone (clear zone/ halo zone) surrounding the colonies, which indicates the breakdown of CMC-Na by the fungal isolates via the release of cellulase³⁹⁻⁴¹

Hydrolyzing index (HI)

The fungal isolates with cellulolytic activities were selected, and their hydrolyzing index (HI) was calculated. The HI is also termed as the Index of Relative Enzyme Activity expressed as the ratio of the hydrolyzing zone diameter to the colony diameter.⁴²⁻⁴³ The greater HI value corresponds to the higher cellulase production/ enzymatic activity of the fungus.

Hydrolyzing index (HI)=(Hydrolyzing zone diameter)/ (Colony diameter)

Identification of Cellulase-Producing Fungi

The fungal isolates confirming cellulase activity were identified by examining the morphological characteristics with the help of microscopic images up to the genus and species level using various fungal identification manuals⁴⁴⁻⁴⁵ and fungal identification databases such as http://www. indexfungorum.orgn and http://www.mycokey.com.

Results

Biomass Loss Pattern

After 360 days of the decomposition period, the lowest amount of biomass (13.97 %) was recorded for shredded leaf litter, followed by intact (14.70%), and the control showed the highest biomass (71.70%), as shown in Figure 1.



Experimental Treatments

Fig. 1: Amount of MF leaf litter biomass remaining in control, intact and shredded treatments after 360 days. (T-bars on the top of the histogram represent the SD of means).

The extensive study of the effect of shredding MF leaf litter using the modified litterbag method showed a three-phased pattern of biomass loss for both intact and shredded states. Also, a strong positive correlation was observed between biomass loss with time for intact (r^2 =0.936; p =0.001) and shredded (r^2 =0.935; p =0.001) MF leaf litter. In the first phase of the study, i.e., upto120 days period, a rapid decline in biomass was observed, and 50% of the total

litter biomass was lost for both intact and shredded states [Figure 2]. However, the second phase of decomposition from 120-180 days displayed a very slow rate for both intact and shredded MF leaf litter. During the third phase of decomposition, between 180 to 360 days, both intact and shredded MF leaf litter showed a rapid loss of biomass, as shown by the loss of more than 80% of the biomass.



Fig. 2: Biomass loss pattern after 360 days: (a) Intact leaf MF litter and (b) Shredded MF leaf litter. (*p= 0.001)

Based on the biomass loss, the rate of decomposition was calculated (Table 1), and the shredded MF leaf litter showed a slightly higher value $(0.029 \pm 0.016 \text{ g/} \text{ day})$ than the intact state value $(0.028 \pm 0.015 \text{ g/day})$.

Table 1: Biomass	Decomposit	ion rate	(g/day)
of Intact and	Shredded MI	F leaf litt	er.

Biomass Decomposition Rate (g/day)			
Intact (±SD)	Shredded (±SD)		
0.028±0.015	0.029±0.016		

Percentage of Nutrients Released

The soil in the experimental plot was slightly acidic pH (6.13 ± 0.79) , and the total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) contents were 2.31 ± 0.30 %, $0.15\pm0.02\%$ and $0.13\pm0.06\%$ respectively. The percentage of nutrients released after 360 days of decomposition was calculated for intact and shredded MF leaf litter with respect to their initial biomass, nutrient content and remaining biomass. The total organic carbon (TOC%), total nitrogen (TN%) and total phosphorus (TP%) release followed a similar pattern (TOC>TN>TP) for both

intact and shredded MF leaf litter (Table 2). However, it was observed that the shredded MF litter showed a marginally higher amount of release of nutrients than intact MF litter.

Table 2: Percentage of nutrients released by
intact and shredded MF leaf litter at the end
of 360 days of decomposition.

Parameters	Nutrients Released (%)			
	Intact	± SD	Shredded	± SD
TOC (%)	86.80	3.88	87.20	4.10
TP (%) TN (%)	86.60 75.10	1.71 2.46	88.54 77.11	0.32 6.33

Annual Decay Constant (k)

The decay constant of MF leaf litter was calculated at the end of 360 days with respect to the final remaining biomass. It was observed that the shredded leaf litter had the highest k value (1.99) as compared to the intact (1.92), while the control had the lowest k value (0.33). The comparison of the k, t_{50} and t_{99} values with other avenue tree species shows that intact MF leaf litter (control) has the lowest k value (0.33), suggesting that it will require

the longest time (t99) to decompose 99% of its biomass in natural conditions (Table 3).

Type of leaf litter	Decay Constant (k)	t ₅₀ (year)	t ₉₉ (year)	References
Intact MF (Control) Intact MF	0.33 1.92 1.99	2.08 0.36 0.35	15.03 2.60 2.51	Present study
MF	0.80	0.86	6.22	Arunachalam and ND Singh, 2004
Tectona grandis Pinus kosivo	1.27	0.54	3.92	Jha, 2010 Arupashalam, 1008
Quercus dealbata	0.88	0.80	5.70	Alunachalam, 1990
Quercus griffithii	1.39	0.50	3.60	
Rhododendron arboreum	0.77	0.90	6.50	
Sterculia khasiana	1.06	0.70	4.70	

	Table 3: Decay constant (k),	t50 and t99 values for MF leaf litter and	other avenue tree species.
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Isolation, Screening and Identification of Cellulase-producing Fungi

A total of 170 fungal colony-forming units (CFUs) were isolated from decomposing MF leaf litter

samples during the decomposition period. Eighteen (18) isolates showed cellulose-degrading activity, as indicated by the presence of hydrolyzing zones on the culture plates (Table 4).

SI. No.	lsolate No.	Genus/ Species	HI (±SD)	Cellulase activity
1.	F-2	Penicillium sp.1	1.42 ± 0.05	+
2.	F-7	Penicillium sp.2	1.31 ± 0.02	+
3.	F-8	Penicillium brevicompactum	1.46 ± 0.01	+
4.	F-15	Mucor sp.	1.17 ± 0.00	+
5.	F-16	Penicillium sp.3	1.30 ± 0.09	+
6.	F-20	Unidentified 1	1.28 ± 0.16	+
7.	F-39(A)	Penicillium sp.4	3.54 ± 0.47	+++
8.	F-60	Penicillium sp.5	1.34 ± 0.08	+
9.	P-2/F-11	Unidentified 2	1.20 ± 0.06	+
10.	P-2/F-28	Penicillium decumbens	2.60 ± 0.57	++
11.	P-6/F-15(A)	Aspergillus <i>niger</i>	4.25 ± 0.25	++++
12.	P-6/F-15(B)	Penicillium chrysogenum	1.09 ± 0.06	+
13.	V-3/F-3	<i>Fusarium</i> sp.	1.09 ± 0.04	+
14.	V-3/F-4	Penicillium sp.6	2.70 ± 0.36	++
15.	V-3/F-17(A)	Penicillium perpurogenum	3.06 ± 0.42	+++
16.	V-3/F-22	Penicillium sp.7	1.95 ± 0.49	+
17	V-3/F-25	Penicillium variabile	1.62 ± 0.03	+
18.	BR-II-A/5	Unidentified 3	1.35 ± 0.05	+

Table 4: List of fungal isolates showing cellulase degrading activity. (low activity = +, moderate activity = ++, high activity= +++/++++)

The identified fungal isolates belonged to 4 genera: Aspergillus, Fusarium, Mucor and Penicillium. Six (6) species were identified up to the species level, 9 were identified up to the genus level, and 3 were unidentified species. Aspergillus niger showed a very high HI value of (4.25 ± 0.25) followed by Penicillium perpurogenum and Penicillium sp. 4 with an HI value of (3.54 ± 0.47) and (3.06 ± 0.42) respectively. Penicillium sp.5 and Penicillium decumbens showed moderately high HI values (2.70 ± 0.36) and (2.60 ± 0.57) , while the rest of the isolates showed lower HI values.

Discussion

A considerable amount of leaf litter is produced by Mesua Ferrea L. (MF) trees growing as avenue plantations throughout the year. The leaves are recalcitrant to the natural decomposition process and remain for a long time on the surface of the ground. The decomposition of MF leaf litter in natural conditions using the modified litterbag embedding method revealed that shredded leaf litter showed a slightly higher decomposition rate as compared to intact leaf litter. In contrast, leaf litter under controlled conditions (unembedded) has shown a significantly slower decompaction rate, as revealed by a high amount of biomass remaining undecomposed over 1 year. In the present study, it was found that significantly lower amounts of biomass remained at the end of 360 days, as shown by shredded (13.97%) and intact (14.7%) MF leaf litter. A study conducted on MF leaf litter decomposition in natural conditions by using the modified litterbag method revealed that 46.60% of biomass remained at the end of 360 days.⁴⁶ However, in their study, the embedded litterbags were covered with MF leaf litter as compared to the present study, in which the litterbags were covered with a layer of soil. Rapid decomposition in the litterbags could be attributed to the availability of moisture and the abundance of diverse microbial communities in the soil. The litterbags were embedded during the monsoon month (August) characterized by frequent rainfall, high temperature and humidity. As reported by various researchers, temperature and moisture are the two major factors that affect the decomposition rate.⁴⁷⁻⁵⁰ However, the rate of decomposition was very slow in the case of control treatment where leaf litter was openly scattered on the soil surface, exposed to normal environmental conditions.

The comparative study of the decomposition of intact and shredded states of MF leaf litter in the litterbags showed a marginal difference following threephased decomposition patterns, i.e. initial rapid decomposition up to 4 months (120 days) followed by a slow decomposition phase between 4 to 6 months (120-180 days) and a rapid decomposition phase after 6 months to 12 months (180-360 days). In the first phase of decomposition, the decline in biomass loss was rapid⁵⁰⁻⁵³ could be due to rapid microbial decomposition and absorption of nutrients, especially the water-soluble components and simple substrates.54 The second phase showed no significant biomass loss, which may be due to the recalcitrant fraction (lignin) of the leaf litter.55-56 During the third phase of decomposition, both intact and shredded MF leaf litter showed a more or less uniform pattern of biomass loss up to 360 days, which could be attributed to the acclimatization of microbes capable of decomposing recalcitrant fractions over time. In addition, the decreased decomposition rate during winter could be due to cold and insufficient moisture availability and reduced microbial activities.57-59 The findings of this study assume that the smaller sizes of leaf litter in shredded leaf litter mixed with soil materials could have provided suitable micro-environmental conditions for the growth and reproduction of microorganisms, leading to the degradation of leaf tissues. The rate of decomposition might have been enhanced since most microbial invasions occur near or on the surface of leaf tissues.60 A similar observation of a three-phased decomposition pattern was also reported for MF leaf litter; however, the initial stage of their investigation exhibited a moderate level of decomposition lasting up to 90 days, which was then followed by a gradual decomposition phase lasting up to 180 days and finally a rapid decomposition period lasting up to 360 days.46

Based on decay constant (k) values, the shredded leaf treatment required less time (approx. 0.35 years) to decompose 50% of its biomass as compared to the intact (0.36 years). The k value of the control treatment showed the highest time of 2.51 years required for the decomposition of 50% of the biomass. The time (t) required to decompose 99% of the biomass will be 2.51, 2.6 and 15.03 years for the shredded, intact and controlled leaf litter of MF,

respectively. The k values of intact and shredded leaf litter of MF in this study were significantly higher than the same species and other avenue tree species reported by different studies.^{46,61} According to the nutrient dynamic analyses, the nutrients released were observed in the pattern of TOC>TP>TN for both intact and shredded states. However, each nutrient released value was slightly higher for the shredded leaf litter state as compared to the intact state. A similar study conducted by Jha (2010)⁶¹ on Tectona grandis leaf litter decomposition reported that the nutrients released flow pattern was TOC>TN>TP.

Screening for cellulose-degrading activity revealed that *Penicillium* was found as the dominant fungal genus with 12 isolates. *Aspergillus* niger showed the highest cellulase activity with the HI value of 4.25±0.25, followed by *Penicillium perpurogenum* with 3.06±0.42. Previous studies have also reported cellulose-degrading activities of different species of *Aspergillus* and *Penicillium*, particularly *Aspergillus* niger and *Penicillium perpurogenum*.⁶²⁻⁶⁵

Conclusion

In the present study, the decomposition rate of MF leaf litter has been found to occur at a faster rate by applying the modified litterbag method where the litterbags were covered with a layer of soil. After 360 days of the decomposition period, the embedded litterbags, i.e., shredded and intact MF leaf litter, were found to decompose rapidly as compared to non-embedded leaf litter spread on the surface of the soil as control. The shredded and intact MF leaf litter displayed higher values of decay constant (k), indicating that decomposing 99% of biomass will require 2.5 years and 2.6 years, respectively, using the proposed litterbag method. However, the lower decay constant (k) value of the control MF leaf litter indicated that the decomposition of 99% biomass naturally would require a very long period of approximately 15.03 years. The presence of dominant cellulose-decomposing filamentous fungi like Aspergillus and Penicillium might have played an important role in the faster decomposition of MF leaf litter. Based on the findings of the present study, it can be concluded that proper management of the leaf litter generated by MF in avenue plantations can be accomplished by burying the leaf litter in a suitable area of the avenue plantation premises instead of burning it. The decomposed biomass in the form of compost or manure produced within 4 months may be utilized for the horticultural, floricultural and agricultural soil management practices of campus and other agricultural lands. In addition to enhancing aesthetic values, this sustainable management technique may reduce air pollution caused by burning leaf litter and reduce the need for chemical fertilizers in horticultural and floricultural activities on academic and government premises in the long term.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

There is no additional data available for the present research work.

Ethics Approval Statement

Present study did not involved any experiment on humans or animals.

Author's Contribution

- Nirigi Linggi conducted the experiment, collected and analyzed data and she has also drafted the manuscript of this research work.
- S. Sureshkumar Singh has developed the concept, designed the experiments and edited the manuscript of the present research work.
- Ajay Bharti has also been involved in experimental design, data analysis and editing the manuscript of the research work.

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