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Recycling flower waste to humus rich compost using effective microbial consortium and mechanical intervention

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ABSTRACT

The importance of organic manure as soil amendment is increasing day by day especially for the improvement of soil health. Flower waste has a high moisture content and easily degradable carbon content. A long duration leachate and odour problem arises during accumulation of flowers. The study aimed on changes in the physico-chemical parameters during the pit method composting of flowers using effective microbial consortium. Four different composting mixture-marigold flower waste + Dried Leaves (1:1), Marigold flower waste + Dried leaves (1:1) + Microbial consortium, Rajnigandha flower waste + Dried leaves(1:1) and Rajnigandha flower waste + Dried leaves (1:1) + Microbial Consortium were prepared. Results revealed that the temperature profile in all the treatments in cemented pits showed a rapid procedure from ambient temperature of 32ºC to 55ºC. The maximum temperature obtained was 55ºC within six days in the pits and pH varied from 4.5 to 5.5 and after 30 days it was found to be 7.0. The measurement of humus content was observed more in the treatments where fungal inoculants were applied as compared to un-inoculated treatments. The final product was rich in carbon, nitrogen content and was found non-phytotoxic.

Key words: Humus, Microbial Consortium, Phytotoxic, Self-heating

India is known as land of temples and each temple generates around 100 kg on an average flower waste (called Nirmalya). In addition flower merchants also dump unused flowers and leaves every day in open only. The leachate produced from natural degradation of the flower waste percolates to ground water and may cause several health issues. To avoid all negative aspects it is necessary to adopt a proper recycling process which stabilizes the organic matter and adds value to the end product.

Microbial composting of plant residues involves enzymatic oxidation of the plant cell components, viz. cellulose, hemicellulose and lignin, resulting in transformation of lignocellulose into a stabilized end product named humus (Ryckeboer *et al.* 2003). Determination of both the maturity and stability parameters is important, as application of unstable or immature compost causes phytotoxicity which can be determined through seed germination indexes (Logan *et al.* 1995). Efficient microbial consortium has been demonstrated to rapidly enhance the degradation of waste residues and convert it to compost for its usage as soil amendments (Kumar, Gaind *et al.* 2008, Gaind, Nain *et al*. 2009, Shukla *et al.* 2016).

The filamentous fungi have been extensively studied for their capacity to produce lignocellulolytic enzymes for degradation of plant biomass (Suwannarangsee, Bunterngsook *et al.* 2012). Consortium of lignocellulolytic microorganism acting synergistically for degradation of lignocellulose biomass is necessary (kumar *et al*. 2008). Fungi belonging to *Aspergillus*, *Trichoderma* and *Phanerochaete* are known to decompose lignocellulose residues including agricultural (Gaind and Mathur 2001) and horticultural wastes (López *et al.* 2002).

Studies monitoring compost maturation for lignocellulose based substrates have found significant correlation between C/N ratio for substrates low in lignin content (Parkinson *et al.* 1989). A maturity index is a chronological sequence of maturation during the composting process by transformation of organic matter through the process of humification (Ziarelli *et al.* 2008).

Stability of compost has been determined through estimating chemical parameters (pH, electrical conductivity, C forms, inorganic N forms and cation exchange capacity) and microbiological parameters (respiration and dehydrogenase activity) (Masaguer *et al.* 2003). Presence of starch, hydrogen sulphide, ammonia and nitrates has been reported to be used as rapid tests indicating stability. Monitoring heat dissipated from compost as a result of microbial activity has been monitored determining stage

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of composting process (Weppen 2002).

The purpose of the present study was to determine the ability of the applied microbial consortia in transforming flower waste into humus rich substances for eco-friendly management and to monitor the chemical and biological changes during composting with reference to microflora and enzyme activities. The maturity parameters to determine the quality of compost were also analyzed to evaluate the stability and maturity parameters for recommending it as an amendment for enhancing soil health and a small step towards cleaning the environment.

MATERIALS AND METHODS

Flower residues: Two types of flower waste were collected namely marigold waste consisted of 95% flowers of marigold, 3% rose and 2% Asoka leaves from Shri Sai temple Najafgarh area of New Delhi and the second waste was collected from the IARI fields during 2017-18, having Rajnigandha flowers including bulbs, stems and leaves of the same. The composting mixture were prepared in the following proportions:

1. Marigold flower waste + Dried Leaves (1:1)
2. Marigold flower waste + Dried leaves (1:1)+ Microbial consortium
3. Rajnigandha flower waste + Dried leaves(1:1)
4. Rajnigandha flower waste + Dried leaves (1:1)+ Microbial consortium

Microbial consortia (MC): Microbial consortia comprised of fungi, viz. *Trichoderma longibrachiatum, Dechslera halodes, Eupenicillium crustaceum* and *Paceliomyces variotii*. Preservation and culture of these fungi were carried out using Potato Dextrose medium. Mass production of these cultures were carried out using jaggery medium formulation as mentioned in (Shukla *et al.* 2014).

Experimental setup: The material was put in cemented pits having dimensions of 4×2×1 (L×B×H) in meters in the Division of Microbiology, Indian Agricultural Research Institute, New Delhi and allowed to decompose. Each pit contained 100 kg of composting mixture and as per treatment variables, amendments were in the ratio (amendment: flowers) 1:1 for leaves, 1:4 for old compost and good quality soil respectively. All the pits were filled on the same day and inoculated with microbial consortia. Moisture level of composting mixture was maintained at 75% and turning of composting mixture was carried at 15 days interval. Samples were collected periodically and underwent physicochemical, microbiological and phytotoxicity analysis.

Electric powered turner cum mixer for pit composting: For pit composting a compost turner cum mixer was designed**.** The electric powered compost turner-cum-mixer is a push type manually operated machine used for thorough mixing of material placed in the pit for compost preparation. The main components of the equipment are a mixing rotor, a power transmission system to operate the rotor, and a three phase electric motor mounted on a frame and fitted with two transport wheel for easy movement from one place to other. The power source is a single phase 0.5 hp electric motor (0.370 kW) (Fig 1). The power is transmitted to the turning rotor through gear box with a speed reduction ratio 1:20 which maintains 72 rpm during turning and mixing process. The rotor is made from a stainless steel shaft having a diameter of 25.4 mm and fitted with projections made of stainless steel blades over its body surface and also provided with mounted type screw with a pitch of 10 mm for digging hole into the raw material before starting turning and mixing process for efficient operation of machine. A total of 8 blades arranged in a helical path facilitate thorough turning and mixing of the material. The design takes care of the dynamics of compost handling in the pit in an efficient manner. The rotor mounted on a frame makes it a complete turning and mixing unit. To facilitate safe transport of the equipment, machine is provided with two rubber wheels of size 240 mm and also the rotor unit can be detached before transportation for safety purpose. Total weight of machine is 76 kg.

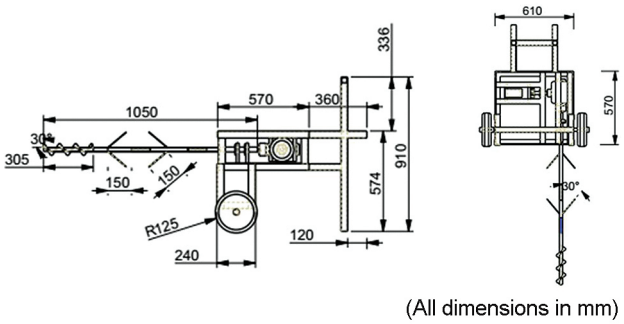

Fig 1 Turner cum mixer for pit composting.

The working depth of the machine is 1.05 m and operational diameter to turn and mix the material is 300 mm through which machine is able to handle 0.074 cubic meter material during turning. In order to get quality compost, three numbers of turning and mixing is required which aerates material and accelerates composting process.

Quality assessment of compost

Physical parameters: Electrical conductivity (EC) and pH of the composted flower waste was determined with the help of hand held electronic EC meter. For this purpose, 10 mL distilled water was added in 2 g of compost sample, stirred for 5 minutes and allowed to stand for 30 minutes following which measurements were taken. Moisture content was measured by taking 5 g sample and kept in a hot air oven at 70ºC. Weight of the dry mixture was taken and moisture content was calculated. Temperature was recorded from the core of the composting mixture with the help of a dial thermometer having 1 m long shaft throughout the experiment and their average values were used for analysis. Ambient temperature was gathered from the institutional weather acquisition centre.

Chemical parameters: Organic carbon was determined by the weight loss method (Walkley and Black 1934). Samples were air dried and sieved through 100 micron mesh, following which 1 g was taken in a silica crucible

and ignited at 600ºC in a muffle furnace for 4 h. The weight of ash content was measured and the organic matter content was calculated using the formula:

$$\text{Organic carbon (\%)} = (100 - \text{Ash (\%)}) / 1.74$$

Total nitrogen content of the samples was estimated by micro-Kjeldahl method. Digestion was followed by distillation process using Kjeltec auto analyzer 1030 used for N estimation. Extent of degradation was determined through estimating humus substances by the method of Kononova (1961).

Microbiological parameters: During the composting of flower waste the changes in the microflora at various stages of composting was monitored. Plate counting method was used to examine population density of cellulolytic microorganisms at different composting stages by subjecting 20 g of subsamples to serial dilution of 10^{-3} to 10^{-7}, spreading an aliquot of 50 µL dilution on cellulose-congo red agar, and incubating the plates at 30 ± 2ºC for 3–4 days to obtain colonies of cellulolytic microorganisms. The activity of cellulase was measured according to the method of Teather and Wood (1982). Similarly, various multi functional bacteria like amylolytic on starch agar, lipolytic on Tributyrin agar, proteolytic on Milk agar, Gram positive on Crystal violet, Gram negative on Methyl red and fluorescent pseudomonads on King's B agar medium were also enumerated.

Phytotoxicity assay: A germination bioassay was used as an indicator of compost maturity and phytotoxicity. Hundred seeds of *Lepidium sativum* seeds were sown on filter paper and moistened with water extracts of compost products. For control distilled water was used to moisten filter paper for seed germination. After 5 days incubation at 25ºC, germinated seeds were counted (G) and the radicle growth (L) measured, and germination index (GI) was obtained according to Eq. (2), where, G(0) and L(0) were values obtained using distilled water.

$$GI = [(G_e \times L_e) \backslash (G_c \times L_c)] \times 100$$

where: GI – seed germination index in percentage, G_e – number of seeds germinated using compost extract, L_e – length of radical of germinated seeds treated with compost sample extract, G_c – number of seeds germinated using distilled water, and L_c – length of radical of germinated seeds treated with distilled water.

All the determinations were carried out in triplicate and CD values at 5% were used to find the significant differences between the treatment means.

RESULTS AND DISCUSSION

Changes in temperature during composting: The temperature profile in all the treatments in cemented pits showed a rapid self-heating procedure from ambient temperature of 32ºC to 60ºC (Fig 2). The temperature reached peak values after 3–6 d in different treatments. The temperature in all the pits started rising after day one of pit preparation. The rise in temperature was monitored and maximum of 60ºC was obtained after first 6 days in the treatments where microbial consortium was applied - T2 and T4 for both type of flowers as compared to uninoculated treatments - T1 and T3 and after peak value, it remained stable for another 4 days and then started decreasing. Temperature varied from 40ºC to 60ºC. As it is known that for effective composting, the presence of both stages mesophilic as well as thermophilic are important and hence after every 7 days interval the pit was given a turning where the whole material was mixed thoroughly within the pits and care was taken not to add anything more in the pits except the moisture, this also maintained aerobic composting. Due to turning, the non-degraded and degraded material mixed thoroughly and when turning was given on the 8th day, microbial activity accelerated and enhanced degradation and also resulted in another small thermophilic phase of 45ºC which was observed for 4 days and then declined gradually towards ambient level. The temperature in compost materials inoculated with ligno-cellulolytic microorganisms was constantly higher than in other treatments, indicating higher level of microbial activities and faster decomposition rate in the inoculated systems. The differences were significant especially during the thermophilic stage, the highest temperature; 60ºC was detected in the treatments with fungal inoculation, whereas that in others remained below 55ºC. The sequence of the temperature in different treatments was as following: T2 and T4 > T1 & T3. The temperature of composting substrates is the reflection of biological degradation rate and plays a selective role on the evolution and succession of the microbiological communities. In terms of degradation and composting, the operating temperature ranges are as follows: >55ºC to maximize removal of pathogens, 45–55ºC to maximize the biodegradation rate, and 35–40ºC to maximize microbial diversity. The results showed that the composting with lignocellulolytic fungal inoculation increased retention of the higher temperature ranges suggesting a maximized removal of pathogenecity and biodegradation.

Moreover, the temperature curve of the composting also indicated that the compost start to become mature after 30 days of initiation as evidenced by their inability to reheat under condition of optimal moisture and aeration.

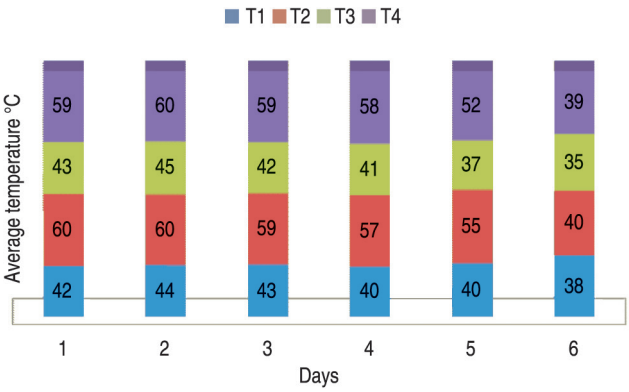

Fig 2 Changes in temperature during decomposition process recorded for 60 days.

Table 1 Changes in organic carbon, total N and C:N ratio during degradation of flower waste

Treatment	Organic C % (Days)				Nitrogen % (Days)				C:N			
	0	15	30	45	0	15	30	45	0	15	30	45
T1: MFW+DL	40.9	39.7	37.1	34.8	0.8	0.9	0.9	1.0	51.1	44.1	41.2	34.8
T2: MFW+DL+MC	41.2	40.3	38.6	32.1	0.8	1.1	1.2	1.4	37.4	33.5	32.1	22.9
T3: RFW+DL	41.0	39.8	37.4	36.1	0.79	0.8	0.85	0.9	47.0	45.8	39.3	35.7
T4: RFW+DL+MC	41.1	37.9	35.5	32.2	0.8	1.0	1.2	1.3	49.6	40.7	33.1	24.7

Changes in pH, moisture content, organic carbon and total nitrogen

The range of initial pH in pits was from 4.5 to 5.5 and it started dropping till 7 days and reached less than 4.0 within 7 days and after 7 days again it started increasing and a maximum of 7.9 was observed after 30 days (Table 4). There was not much variation in pH among the four treatments. Electric conductivity: An initial EC 3.8 dS/m was observed and as the degradation progressed EC value 4.8 dS/m was recorded up to 45 days with no variation among the treatments. The initial moisture content was recorded 60.8% and after 15 days it increased to 68.9 % and later in final product it decreased to 45.2% in all the treatments. The results showed that the fungal inoculants had no remarkable effect on the moisture content.

Changes in chemical characteristics: The changes in organic carbon (C), total nitrogen (N), C:N ratio varied considerably during degradation of two types of flowers. Table 1 shows the changes in total organic carbon during the composting of both types of flower wastes. The initial organic carbon in all the four treatments including treated and untreated varied from 34.8% to 42.2%. The content of organic carbon decreased as the degradation progressed and different treatments had different carbon mineralization rates, and the differences were significant during the rapid phase as evidenced by the drastic decreasing of organic carbon at the first 15 days for the treatments with fungal inoculation; whereas for the treatments without inoculant, the decreasing of organic carbon at the same period was recorded slightly lower. At the end of the experiment, it was seen that the lowest amount of organic carbon was present in marigold inoculants treated waste as compared to untreated and similarly for rajnigandha waste which clearly indicated the role of inoculated fungi for degradation. Soluble carbohydrates and amino acids were the major sources of C for the microorganisms involved in the first period of decomposition, while the decomposition of polysaccharides including cellulose mainly occurred in the cooling and maturing stages of the composting.

At the start of composting the total nitrogen content of untreated and treated waste material was same but at the end of the degradation total nitrogen was higher in treated treatment for both types of flower waste and 1.4% was recorded for marigold and 1.3% for rajnigandha flower waste respectively. There was no decrease in total N loss as ammonia at any stage of degradation and increase in nitrogen content was observed with increase in time.

The carbon content of the compostable flower waste material decreased with time and Total N content increased which simultaneously resulted in decrease in C:N ratio. As the time progressed, the decrease in C:N ratio was found more in the treatments where fungal inoculants were applied (T2 and T4) as compared to the uninoculated treatments (T1 and T3). Table1 shows the changes in C: N ratio during degradation. Both types of flower waste degraded within 45 days and the initial C: N ratio used for composting ranged from 37.4 to 51.1. As the decomposition progressed the loss of carbon was mainly due to CO_2 evolution. After 45 days of degradation, the C:N ratio of the end product varied from 22.9–24.7 in the treatments with fungal inoculants and 34.8–35.7 in untreated treatments indicating that more time was needed for degradation by the two treatments (T1 and T3).

Population dynamics of microorganisms

Microbial succession plays a key role in degradation process and appearance of some microorganisms reflects the quality of maturing compost (Ishii *et al.* 2000, Ryckeborer *et al.* 2003). The samples were analyzed for total bacteria, fungi and actinomycetes population during the degradation of both types of flower waste material. It was observed that there was an increase in total microbial population in all

Table 2 Enumeration of microbial population during degradation of flower waste

Treatment	Bacteria (×108 cfu/g soil) (Days)			Fungi (×103 cfu/g soil) (Days)			Actinomycetes (×104 cfu/g soil) (Days)		
	0	15	30	0	15	30	0	15	30
T1: MFW+DL	241	264	260	8	11	13	30	62	38
T2: MFW+DL+MC	226	284	293	10	27	31	28	79	36
T3: RFW+DL	221	232	240	5	9	7	25	60	25
T4: RFW+DL+MC	220	256	284	4	28	30	28	70	32
CD (P=0.05)	11.85	24.129	6.738	2.208	4.204	5.351	4.998	14.372	7.025

Table 3 Changes in protease, xylanase and cellulase activity during degradation of flower waste

Treatment	Protease (mg reducing sugar/kg dry matter/hr)				Xylanase (mg reducing sugar/kg dry matter/hr/)				Cellulase (mg reducing sugar/kg dry matter/hr/hr)			
	0	15	30	45	0	15	30	45	0	15	30	45
T1: MFW+DL	91	112	118	82	24	39	51	112	27	47	64	97
T2: MFW+DL+MC	92	120	139	98	27	40	87	129	29	59	84	110
T3: RFW+DL	91	110	124	87	25	38	45	84	25	42	59	64
T4: RFW+DL+MC	93	115	135	92	26	42	94	120	26	58	74	101
CD (P=0.05)	NS	3.703	4.484	2.868	NS	4.130	5.575	5.492	3.218	4.204	4.585	5.089

the treatments with and without fungal inoculants (Table 2) but with varied load which ranged from 220-293 $\times 10^8$ cfu/g soil for bacteria, 8-12 4-30 $\times 10^3$ cfu/g soil for fungi and 25-79 $\times 10^4$ cfu/g soil for actinomycetes, respectively (Table 2). The increase in microbial population was observed till 30th day and a sharp decline was observed after that but wherever fungal inoculants was applied (T2 and T4), the fungal population was much higher as compared to untreated treatments (T1 and T3).

The population dynamics of cellulolytic, amylolytic, pectinolytic, lipolytic, diazotrophs, fluorescent pseudomonads during degradation of flower waste was also studied. In two treatments T1 and T3, except for T2 and T4 with fungal inoculation, the numbers of the functional microbial population were suppressed by high temperature at the thermophilic stage, then increased gradually during the mesophilic stage, and obtained a peak at the cooling and maturing stages. The number of cellulolytic microorganisms was constantly and significantly higher in the treatment with fungal inoculation than that of other treatments, which means that the initial inoculation significantly enhanced the population density of cellulolytic microorganisms. The initial inoculation of fungal culture at the time of start showed no significant effect on population density of cellulolytic microorganisms during the thermophilic stage, which means the added ligno-cellulolytic fungi were partially killed or inactivated during the thermophilic stage. These results help in the conclusion that although the availability of nutrients may play an important role in the development of the total microbial biomass over time, different subsets of microorganisms were clearly selected by the temperature regime during the composting process as has also been observed by Klamer and Baath (1998).

Hydrolytic enzymes during degradation process

Cellulases, hemicellulases, xylanases, proteases are few hydrolytic enzymes which are thought to control the rate at which substrate is degraded. Enzymes are the mediators of various degradative processes (Mckinely *et al.* 1985, Tiquia *et al.* 1996, Tiquia 2002), hence the changes in the activities of these three main hydrolytic enzymes cellulose, xylanase and protease were studied during degradation of both types of flower waste. The cellulose activity of treatments where fungal inoculants was applied (T2 and T4) showed an increase in activity from initial till 30th day of degradation and it was found maximum on 30th day and declined by 45th day of degradation (Table 3). As the maximum cellulose activity was recorded for both the treatments where fungal inoculants was applied (T2 and T4) clearly indicates that cellulose activity depends on types of cellulolytic microflora activated during degradation, whereas the treatments without fungal inoculants showed lower amount of activity. Similar trends were recorded for xylanase activity (Table 3) while protease activity increased with degradation and the maximum activity was observed on 45th day and the activity varied from 82-98 mg reducing sugar released dry matter/kg/h (Table 3) and further the results revealed that like earlier enzymes protease activity was much higher in inoculated treatments (T2 and T4) as compared to uninoculated treatments (T1 and T3). Cunha Queda (2002) also determined the activities of cellulases, lipases and proteases in composts prepared from various substrates like pig slurry, horse manure, cardboards and observed lower activities as compared to flower compost.

Changes in humus content during the degradation of flower waste

Humus content of the samples from different treatments was assayed following konnova's method and it increased from the initial level of 0.5 to 4.1% as the composting progressed, demonstrating the humification of the organic matter took place during composting. Humus content of the compost with the treatment having flower waste, dried leaves, and fungal inoculation was significantly higher than the un-inoculated treatments (T1 and T3) (Table 4).

Humic and fulvic acids are components of the humic fraction of organic matter. They are generated by the polymerization and condensation of quinines, phenolics or sugars with nitrogen compounds. Humic acid is a more polymerized compound of the humic fraction, and

Table 4 Compost maturity parameters

Treatment	pH	EC	K %	S %	Humus %	Seed germination %
T1: MFW + DL	7.9	2.4	0.6	0.16	1.8	60
T2: MFW + DL + MC	7.4	3.1	1.0	0.20	3.9	100
T3: RFW + DL	7.2	4.2	0.5	0.15	1.0	50
T4: RFW + DL + MC	7.0	3.0	1.0	0.22	4.1	100

consists of heteropolycondensates of phenolic substances. Experimental results demonstrated that the activity of highly effective ligninolytic and cellulolytic microorganisms could significantly contribute to the high biodegradation of the flower waste.

Phytotoxicity analysis and quality control of compost

Compost, a value added product, is the end product of composting and its stability is often related with the compost's microbial activity, whereas maturity is associated with plant-growth potential or phytotoxicity. It relates to the degree to which the organic matter has stabilized during degradation (Eggen and Vethe 2001, Weppen 2002). The result of germination test with *Lepidum sativum* seeds showed that all the samples taken at the maturation period had GI values of greater than 50%, which, according to Zucconi *et al.* (1981) indicates a phytotoxin-free compost product. Table 4 shows that all the germination rates treated with 1% extraction of composting products were above 95%, indicating the compounds present in the raw wastes or produced during the first day of composting as intermediate products of microbial metabolism, were degraded within 30 days of composting. Requena *et al.* (1997) reported that inoculation with the selected microorganisms and further incubation of lignocellulosic wastes can be a useful tool for the improvement of the agricultural value of the resultant product, i.e. compost, probably as these methods render nutrients more available to plants. This also confirms the result of our research work as evidenced by a higher germination rate and index when the seeds were incubated with the extracts of the compost with fungal inoculant and dried leaves as compared with the other two treatments. All the samples were also tested for the presence of starch, sulphides and ammonia using rapid tests. It was found that the treatment where microbial inoculation was done resulted in absence of all the three elements indicating the ripening of compost (Inbar and Hadar *et al.* 1993) while the treatment where no microbial inoculation was done showed the presence of starch, ammonia and sulphides indicating the semi-degradation of raw material after 45 days. The composting process results in the production of humic substances which are slowly degradable and have the stability to improve soil physical and chemical properties (Chen *et al*. 1996). The humus content was observed to be more in the treatments where fungal inoculants was applied (T2 and T4) as compared to un-inoculated treatments (T1 and T3) and can be considered as source for the improvement of soil properties.

The study demonstrated that the addition of the mixed inoculant of ligno-cellulolytic fungal microorganisms and addition of dried leaves in nirmalya or temple flower waste as well as in rajnigandha flower waste enhanced degradation rate, and also improved the physical and nutrient properties of flower compost. The study also indicated that these ligno-cellulolytic microorganisms can be considered as potential inoculum for composting the Nirmalaya, the holy waste in an acceptable manner where no application of earthworms and cowdung is involved and goes with the sentiments of people. Further, the maximum hydrolytic enzyme activities on 30th day indicates the active phase of degradation and decrease in C:N ratio can be taken as an index of compost maturity along with seed germination and humus content.

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