Composting of floral waste by using indigenously isolated microbial consortium: An approach towards the Environment sustainability and waste management

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Abstract— *In India huge number of flowers are offered in temple creating a large amount of flower waste. The temple waste is released in the water bodies or dumped at the available places of land which creates severe environmental pollution and health hazards. Floral waste is biodegradable and contains elements required for growth of microorganisms. The present study focused on the use of Temple floral waste extract for preparation of microbial nutrient media in order to cultivate bacteria (pH7.4) and fungi (pH 5.4). Soil sample was used for screening of microorganisms capable of degrading the floral waste. Thus, in the present study instead of using conventional microbial media we have used the flower waste media to develop microbial consortium for degradation of floral waste. On the basis of capability to produce variety of hydrolytic enzymes two sets of consortia were developed and tested for development of compost as against the control without the microbial consortia. Physicochemical analysis of mature compost revealed that floral waste compost prepared by using the microbial consortium is enriched with the Nitrogen, Phosphorus, Potassium, Calcium and Magnesium. The mature compost developed using the microbial consortia has the potential to support the growth of tomato plants. This method is cost effective as well as pollution free. Thus, it can be promoted as potential mechanism to maintain the environmental sustainability at wider scales.*

Keywords— Compost, Floral waste agar media, Microbial consortia, Temple waste.

I. INTRODUCTION

Waste is defined as unwanted, unusable material and is regarded as a substance which is of no use. Waste disposal is the major concern of the society. If it is not disposed properly that leads to deterioration of environment. Industrial, commercial, agricultural and domestic are different sources of waste generation.

Floral waste is generated by various sources like Temple waste, marriage ceremony, hotels, various others cultural and religious ceremony. Every day the flowers offered in the temples or used in the various ceremony or functions left unused and later on it converted in to waste and it becomes the major constituents of municipal solid waste (Singh et al. 2013). Most of this waste is generally neglected and get added to water bodies which lead to water pollution.

By considering the characteristics of floral waste as it is rich in organic compound, macronutrients and micronutrients, composting is the best solution for proper disposal of floral waste. Composting is defined as the natural biological decomposition of organic matter under self heating, aerobic and moist conditions to produce a stable nutrient enriched product which is used as organic manure (Bustamante et al.2009). Thus floral waste can be converted in to agriculturally useful organic fertilizers which in turn have the potential to reduce the dependency on non-renewable chemical fertilizers and pesticides. Most of the time during the process of composting bulking agents like wood shaving, dry leaves, newspaper, wheat bran, Pumice are added to support the free passage of air during the composting. In this study we have added cow dung as a bulking agent (Sharma et al., 2017)

Degradation of floral waste is a very slow process as compared to kitchen waste degradation (Jadhav et al., 2013). By considering this we have made attempt to develop consortia having potential to degrade the floral waste. The exploitation of the metabolic versatility of microorganisms is advantageous in biological waste treatment this led to shortening of the time required for composting.

In this study, attempt was made to design the successful composting system by using the microbial consortium isolated by using the floral extract agar. Further the potential of floral waste compost to support the growth of plant was studied by pot assay.

II. MATERIALS AND METHODS

2.1 Sample collection (floral waste and soil sample)

Floral waste was collected from following selected temples from Baramati, Siddhivinayak temple at Sayali hills, Tuljabhavani temple, Siddheshwar temple, Ganapati temple at Kasaba and Kashiveshweshwar temple. The collected floral waste was brought to laboratory in polythene bags. The collected FW comprised of the flowers namely Marigold, Rose, Jasmine, and Lotus. In this study, only FW was used for composting without stems, roots and leaves. Soil samples were also collected from above mentioned different temple area for isolation of microorganisms capable of degrading the floral waste.

2.2 Extract from flower waste

After collection of floral waste from different temples, non-biodegradable part i.e plastic, paper, thread and other waste materials were removed by hand sorting and the biodegradable waste i.e garlands and flowers were segregated. The segregated floral waste was air dried by spreading over paper for 48 hours. The air-dried samples were then crushed using the using mixer grinder and made 300 ml paste of flower waste. Again, the homogenized mixture was prepared in mixture grinder. Then this mixture was allowed to stand without disturbance for 3 hour so that debris, if present get settle down. The clear supernatant was filtered through the muslin cloth. The filtrate obtained was referred as a floral extract.

2.3 Media preparation by using Floral waste

Original pH of the floral extract was 4.7, being too acidic, it was not suitable for cultivation of common bacteria and fungi, so pH was adjusted to 7.2 and 5.6 in order to support the growth of bacteria and fungi respectively. For solidification of media, 3.0 g/ 100 ml of agar powder was added in the floral extract, followed by media sterilization at 15psi 121 0C for 30 minutes.

2.4 Screening of floral waste degrading microorganisms

Soil samples were collected from above mentioned temple areas. 0.3 gm of each soil sample was inoculated in to 100 ml of floral waste media. These flasks were incubated at 28 ^{0}C at 125 rpm for 3 days. Flasks were allowed to stand undisturbed for 2 hour so that all the debris gets settled down. Serially diluted supernatant was spread on floral waste media. Plates were incubated at 28 ^{0}C till growth was observed in the form of colonies on agar plates (Jadhav et al., 2013).

2.5 Hydrolytic Enzyme Screening and Assay from isolates grown on floral waste agar

All the isolates were analyzed for their ability to produce different hydrolytic enzymes. All the isolates were grown on the respective substrate (1%, w/v, starch, cellulose, casein, colloidal chitin &pectin) containing agar plate for the determination of amylase, cellulase, protease, chitinase, pectinase activity. The plates were incubated for 5 days and detected enzyme activity. After addition of gram's iodine on starch agar development of halo zone around the colony indicate the amylase positive test. For detection of cellulase activity 0.1% Congo red was added followed by addition of 1M NaCl solution. Protease positive isolates shows development of halo around the colony. For detection of chitinase activity colloidal chitin agar was used Pectinase positive strains were detected by adding iodine solution on pectin plates (Naif et al; 2019).

For induction of above mentioned enzymes the selected isolates were inoculated in the minimal medium containing the following different substrate at 1 % (w/v) starch, cellulose, casein, colloidal chitin & pectin. Flasks were incubated for 5 days at 28^{0}C followed by centrifugation at 10,000 rpm at 4^{0}C for 10 minutes. The supernatant was used for enzymatic assay. Amylase and cellulase activity were determined by DNSA method. Protease activity of culture supernatant was assayed using casein (1%) as a substrate. The substrate was prepared in Tris buffer (pH8.0,50 mM). Then, 1.0 mL casein was mixed with 0.1mL culture supernatant and incubated for 30 min. Then, 10% (w/v) trichloro acetic acid (5 mL) was added and incubated for 30 min. It was centrifuged at 5000 rpm for 5 min and the supernatant was measured at 275 nm against reagent blank using a UV-visible spectrophotometer. L-tyrosine was used as the standard for the determination of protease activity. Pectinase activity of the sample was determined using pectin as a substrate. About 9.8 mL pectin (0.5%) was mixed with 0.2 mL culture supernatant. It was incubated for 10 min and 1 mL sodium carbonate (1 M), 5 mL iodine (0.1 N) were added and kept for 5 min. To this, 2 mL sulphuric acid (2 N) was added and kept in the dark for 15 min. The developed dark colour was further titrated against sodium thio sulphate (0.1 M) using soluble starch (1%) as an indicator. Chitinase activity of the culture supernatant was determined using colloidal chitin as a substrate. This substrate was prepared at the 1% level using 50

mM acetate buffer (pH 5.5). A 1.0 Ml substrate was mixed with a 0.1 mL sample and incubated for 30 min. After that, 1.0 mL DNS was added and further assay was continued following a similar procedure to the cellulase assay (Naif et al; 2019).

2.6 Development of consortia

Two different combinations of four bacterial isolates were prepared and used for composting of floral waste. A loopful growth from 24 hours old bacterial cultures of different organisms in selected combination were inoculated in broth prepared using flower waste. Broth was incubated at 28 ^{O}C for 48 hours. This mix culture was used as consortium and then 25% (v/w) of this consortium as inoculums was added to the flower waste (Pindi and Satyanarayana, 2012)

2.7 Floral waste composting

All organic waste materials were available in form of flowers were collected. As fleshy, pulpy waste material decompose very rapidly and it favors the compost formation, hence collected floral waste was crushed and converted in to small pieces which enhances rate of formation of compost. As moisture is necessary for the growth of microorganisms, soil was added to the floral waste which has capacity to absorb the moisture to support the growth of microorganisms. Then cowdung was added in 1:1 proportion. Four plastic chambers having holes at bottom and side walls for aerations and excess water removal were selected. To maintain the aerobic conditions at the bottom of the chamber layer of coconut coir 2cm height was prepared, it was covered with garden soil. On the top of soil, floral waste along with 25% consortium was added. Chamber filled up with alternative layer of soil and consortium inoculated floral waste were kept at moist and dark place for composting purpose.

Four different chambers were prepared having the following combinations

Chamber 1 Soil +Floral waste (Control1)

Chamber 2 Soil+ Floral waste + cow dung (Control 2)

Chamber 3 Soil+ Floral waste + cow dung + consortium 1 (Test 1)

Chamber 4 Soil+ Floral waste + cow dung + consortium 2 (Test 2)

2.8 Analysis of physicochemical properties of compost

Physical and chemical properties including temperature, pH, electrical conductivity (EC), total organic carbon, total organic matter, total nitrogen, total phosphorus, total potassium and C/N ratio were analyzed. For estimation of pH15g compost was mixed with 30ml of distilled water and kept on rotary shaker for 1 hour. Filtration was carried out and pH of filtrate was checked by using pH meter. Electrical conductivity of the filtrate was measured by using conductivity meter. For estimation of moisture, 5 gm of prepared compost was taken on a dry petri-dish and dried in an oven at 55^{0}C till constant weight was achieved then percentage moisture level was calculated. Compost was diluted in the ratio of 1:10 (w/v) and kept on rotary shaker at 150 rpm for 45 min. This sample was used for further analysis of compost. Nitrogen content was estimated by Kjeldahl method whereas organic carbon content was detected by the method of Walkley and Black (1934).Heating digester (Velp scientifica DK 20) was used for digesting the 0.2 g sample, 10 mL H2SO4 and HClO4 mixture (5:1) at 300°C ± 5°C for two hours. The digested samples were used for the determination of total phosphorous using stannous chloride methods (Adhikari et al., 2009). The concentrations of Na, K and Ca were determined by using flame photometer. The Ca and Mg contents of the samples will also be analyzed by using atomic absorption spectrophotometer. The C: N ratio will be calculated from the measured values of C and N (Maiti, 2003).

2.9 Analysis of plant growth promoting activity of compost

Twenty-five, Tomato plant seeds were sterilized and prepared as per the protocol given by Indananda et al. 2010. After germination of seeds, mature compost was added to the pots. Control set was prepared without addition of compost. After 35 days of treatment plants were analyzed with respect to root length, shoot length and weight of plant

III. RESULTS AND DISCUSSIONS

As shown in Fig1. Floral waste were collected and used for preparation of floral extract (Fig.2). Media was prepared by using floral waste extract; diluted soil sample was spread on floral waste agar media. After incubation of plates at 28^{0}C, plates were observed for the presence of colonies (Fig.3). Total 40 isolates were seen on floral waste agar plates. On the basis of their ability to produce different hydrolytic enzymes, from 40 different isolates, grown on floral waste agar plates, 8 were selected for the development of microbial consortia on the basis of their ability to produce different hydrolytic enzymes.

FIG 1: Floral waste collected from different temples of Baramati

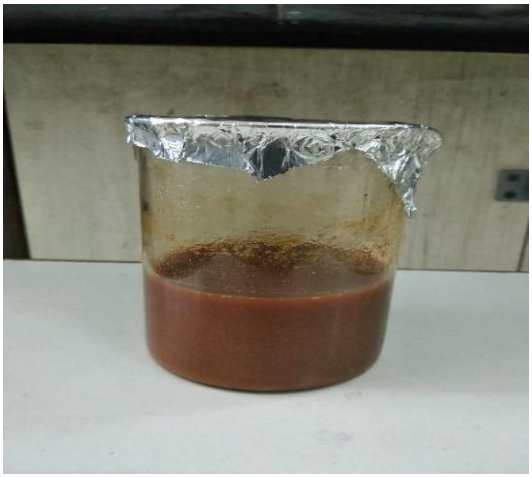

FIG 2: Floral extract prepared from floral waste.

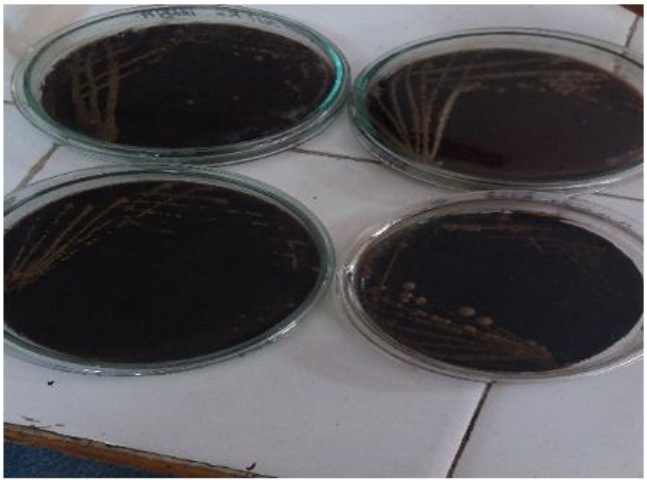

FIG 3: Growth of Microbial isolates on media prepared by using floral extract

TABLE 1

PRODUCTION OF EXTRACELLULAR ENZYMES BY THE VARIOUS STRAINS ISOLATED BY USING FLORAL WASTE AGAR

Name of isolates	Enzyme activity U/ml				
	Amylase	Cellulase	Protease	Chitinase	Pectinase
TW	0.9	34	12	25	1.6
DaA	4	27	39	20	1.3
DaD	12	56	46	34	0.94
KL	9	45	67	27	2.2
KM	12.4	23	98	34	1.9
KS	15	52	84	29	1.7
Min. S	11	48	57	37	1.1
Min. L	10	17	49	31	0.6

Table. 1 depicts the quantitative enzyme activity of selected isolates. All these isolates showed the amylase activity that ranged from 0.9 U/ml to 15U/ml. Cellulase production ranged between 17 U/ml to 56 U/ml. All the isolates were capable of producing protease enzyme. Maximum protease production 98U/ml was shown by KM isolate whereas lowest protease production 12 U/ml was shown by TW isolate. Lowest Chitinase production observed was 20 U/ml whereas highest Chitinase production was 37 U/ml. Pectinase ranged between 0.6 U/ml to 2.2 U/ ml. With this result we concluded that the selected isolates have the potential to degrade organic biomass present in the floral waste with the help of hydrolytic enzyme. This hydrolytic enzyme helps in the formation of compost as they convert complex organic matter in to simpler one. Similar

kind of study was carried out by Gopinath et al., (2014) in order to explore the potential of consortium for effect degradation of organic waste. The enzymes in the selected isolates are intended to start breaking down organic matter to speed decomposition. So that overall time required for compost formation get reduced.

TABLE 2
VARIATION IN THE TOTAL VOLUME CONTENT DURING COMPOSTING

Sr.No.	Days	Height of contain in chamber(in cm)			
		Sample 1	Sample 2	Sample 3	Sample 4
1	1	30	30	30	30
2	3	28	28	27	28
3	5	28	27	26	26
4	7	25	25	25	24
5	9	23	22	23	23
6	11	21	20	21	20
7	13	21	20	19	18
8	15	20	19	14	16

During the composting process overall decrease in the total volume was observed, this variation in the volume is summarized in the Table.2. For Test 1 and Test 2 decrease in volume was respectively 14 cm and 16 cm whereas for control1 and control 2 decrease in volume was 20 and 19 cm at the end of 15 days. This decrease in overall volume was observed because of biodegradation of organic compound. The rapid degradation was observed in Test 1, means consortia 1 is metabolically more active than consortia 2.

Characterizations of Compost obtained in four chambers were carried out and it is represented in Table 3. Physical characteristics of compost revealed that compost formed in Test 1 and Test 2 was of relatively uniform small particles and no big hard chunks were seen. Appearance was dark brown to black with no visible signs of original floral waste. Moisture content was 40%, 35%, 28%, 26 % in control 1, control 2 and Test 1 and Test 2 respectively. Ideally moisture content of compost should be in between 30 to 50%. If it is more than that then compost would be clumpier and if it is less than 30 t0 50% then it would be very dry and dusty.

pH of compost Test 1 and Test 2 ranged between 7.1 to 7.2, it was less than control1 and 2 (7.6 and 7.4) this may be because of the volatilization of ammonial nitrogen and hydrogen ions (H+) release through the nitrification activities of nitrifying bacteria, as well as the emission of large volumes of carbon dioxide (Huang et al. 2004). Similar results were reported for organic waste composting (Kalamdhad and Kazmi 2009). The final pH value between 6 and 8 shows the maturity of the compost (Varma and Kalamdhad 2014a).

TABLE 3
PHYSICAL AND CHEMICAL CHARACTERIZATION OF COMPOST

	Chamber 1	Chamber2	Chamber 3	Chamber 4
Parameter	**Control 1 Soil +FW**	**Control 2 Soil +FW+CD**	**Test 1 Soil+FW+CD+Consortia 1**	**Test 2 Soil+FW+CD+ Consortia2**
Colour	Black	Black	Brownish black	Brownish black
Moisture %	40	35	28	26
pH	7.6	7.4	7.2	7.1
Electrical conductivity mS/cm-1	4.9	4.5	3.4	3.3
Nitrogen (%)	1.67	1.7	1.9	1.85
Organic Carbon(%)	43	45	56.35	54.76
C:N ratio	25.74	26.47	29.65	29.6
P g/kg	3.3	3.9	5.56	5.1
K (g/kg)	16.7	18.7	22.6	20.3
Ca (g/kg)	3.9	4..6	6	5.2
Mg (g/kg)	0.35	0.43	0.54	0.51

Soluble salts in compost are typically measured on a scale of electrical conductivity associated with salt content. Electrical conductivity of Test 1, Test 2 and control 1, control 2 were 3.4, 3.3 and 4.9, 4.5 mS/cm-1 respectively. Electrical

conductivity value shown by the Test 1and Test 2 lie within the range recommended in the literature i.e between 2.0 and 3.5 mS/cm as optimal for using compost as fertilizer in agriculture (Fernández et al., 2007).Conductivity above 5 mS/cm in finished compost can damage roots, affect nutrient uptake, limit plant-available soil water, or cause seed germination to be inhibited (Lim et al., 2016). At low levels, these salts are potentially beneficial minerals that plants can use. It was observed that in combinations 1 and 2 the initial electrical conductivity was 5.44 and 4.87 mS/cm, respectively.

Total nitrogen content of compost formed in Test 1 and Test 2 were 1.9 and 1.85 % and in control 1 and control 2 were 1.67 and 1.7% respectively. Comparatively more nitrogen content was observed in Test 1 and Test 2 this may be because of mineralization of organic matter during the process of biodegradation. Thus the availability of nitrogen is increased which is good to support the growth of plants. These results are similar to those obtained by Benito et al. (2005) who found that the total nitrogen rate ranged from 0.99 to 2.01%.

Carbon to nitrogen ratio (C\N) means the ratio of carbon element in organic matter to the nitrogen element of organic matter. The finished compost of Test 1 and Test 2 revealed the C/N ratio around 29.6 whereas for control 1 and control 2 ratios were 25.74 and 26.47 respectively. According to Singh and Kalamdhad (2013), the best C\N ratio is 20-30 atoms of carbon for each atom of nitrogen (20-30 carbon atoms: 1 nitrogen atom). If the C:N ratio is too low (not enough carbon), the microbes don't have enough available energy to incorporate all the nitrogen into their cells. In that case, the microbes will "eat" all the carbon that's there, but the excess nitrogen will be eliminated as ammonia. If the C:N ratio is too high when land applied, micro-organisms compete with crop plants to consume the available soil nitrogen in order to degrade the carbon in the compost. The resulting nitrogen immobilization may affect the growth of plants.

In the present investigation, the compost showed the Phosphorus content as 5.56 and 5.1 g/kg for Test 1 and Test 2 respectively, whereas 3.3 and 3.9 g/kg respectively for control1 and control 2. Significantly more amount of phosphorus in test was seen as compared to the control this may be because of addition of consortia in test has contributed for rapid degradation of organic matter. The concentration of potassium content was high in case of all the four combinations. 22.6 and 20.3 g/kg respectively for Test 1 and Test 2 whereas 16.7 and 18.7 g/kg for control1 and control2. It shows the high inherent content in FW, suggesting that compost can be a good source of potassium fertilizer.

Calcium and Magnesium are secondary nutrients for the plants, they are required in very small concentration by the plants. Calsium content of compost were 6, 5.2 and 3.9 and 4.6 g/kg respectively in Test 1, Test 2 and control 1 , Control 2. Compost showed the concentration of Magnesium as 0.54, 0.51, 0.35, 0.43 g/kg respectively in Test 1, Test 2, Control1 and Control2.

TABLE 4

EFFECT OF FLORAL WASTE COMPOST ON GROWTH PROMOTING ACTIVITY IN TOMATO PLANT

Period of Treatment	Control/ Test	Root length (cm)	Shoot length(cm)	Total weight of plant in gm
15 days of treatment	Control (1)	1.7	6.2	1.65
	Test(1)	2.4	8.2	2.7
21 days of treatment	Control	2.2	6.9	2.3
	Test	3.1	10.5	4.2
28 days of treatments	Control	2.7	9.8	2.8
	Test	4.1	13.9	4.7
35 days of treatments	Control	3.4	12.3	3.7
	Test	4.7	17.5	5.9

In order to study the effect of floral waste compost on growth promoting activity in tomato plant we have used the compost prepared using consortia group 1 (Test 1). The addition of floral compost resulted in to increase in root length as well as shoot length of the plant as compared to result obtained in control set, result is represented in Table 4. Maximum root length and shoot was 4.7 cm and 17.5 cm respectively. This increased in the root and shoot length was observed from the 15 days of treatment and this trend continue till the 35 days of treatment. Same pattern was observed with respect to total weight of plant. Maximum weight obtained was 5.9 gm as compared to 3.7 gm in control. The observation of growth with respect to the root length, shoot length and weight of plant were in line with the results of Prabha et al., (2007), where scientist had tested the vermin compost on Hibiscus esculentus and Solanum melongena and medicinal plants (Adhatoda vasica and Solanum trilobatum).The overall result revealed the potential of floral waste compost to support the growth of the plant.

IV. CONCLUSION

We could design the successful composting system by using the microbial consortium isolated by using the floral extract agar. Further the potential of floral waste compost to support the growth of plant was studied by pot assay. Thus this process has not only contributed for waste management rather it has provided nutrient rich compost which can be used for variety of plantation. Floral waste compost prepared by using the microbial consortium is enriched with the Nitrogen, Phosphorus, Pottasium, Calcium and Mangnesium. This method is cost effective as well as pollution free. Thus it can be promoted as potential mechanism to maintain the environmental sustainability at wider scales.

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