Physico-chemical and Microbiological Changes in Degradation of Agri-residue under Pit Compositing

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Abstract: Management of crop residues has remained a major challenge due to lack of easily adaptable and affordable interventions at farmer's field. Cellulolytic bacteria and fungi have been recommended for recycling lignocellulosic-rich organic matter and to convert it into nutrient rich compost. However, these inoculants need to be generated on commercial scale for handling large volume of biomass. Also, the microbial formulation should also be capable of degrading diverse type of agri-residues. Seven fungal strains, namely *Aspergillus awamori* (F-18), *Aspergillus nidulans, Trichoderma viride, Trichoderma longibrachitum, Drechslera halodes, Eupenicillium crustaceum* and *Paecilomyces variotii* were characterized based on shelf life, functional activity to make consortium for degradation of various agro and horticultural residues using pit method of composting. An experiment was laid in cemented pits using four different substrates-maize cobs and stalks, paddy straw, kinnow fruit waste and flower waste for rapid degradation. The results revealed a maximum increase in spore count with *Paecilomyces variotii* followed by *Eupenicillium crustaceum* and *Drechslera halodes*. Out of four different additives, glycerol was found to be most suitable additive for incubation of selected fungi. The results on microbial population revealed highest bacterial population in flower waste and highest actinobacterial population in kinnow waste. It was also observed that inoculated organic waste degraded faster than un-inoculated wastes. As the degradation process progressed, total N also increased with time and varied from 1.1 to 1.5 percent. Flower and kinnow waste degradation was completed in 45 days while that of maize and paddy straw in 75 and 90 days, respectively. The pH range was 6.5-8.0 in all the substrates but in inoculated substrates, the range of pH was 7.2-7.6, while electrical conductivity (EC) was 1.4-3.6. The highest humus% was found with paddy straw followed by maize, flower and kinnow. The final product also showed variation in K% and S% which were found to be 0.6% -1.8% and 0.1% -0.20%, respectively.

Keywords: Cellulolytic fungi; inoculant; *In-situ* degradation, agri-residue, consortium

1. Introduction:

During post-harvest processing, major crops in India contribute to heavy amount of residues and byproducts. Cereal crops viz., rice, wheat, maize and millets contribute 70% [1], while rice crop alone contributes 34% to the crop residues. India generates about 620 million tons of the crop residues and rice contributes the highest amount

(154 Mt) residues followed by wheat (131 Mt) [2]. Burning of one tonne of rice straw was estimated for loss of 5.5 kg Nitrogen, 2.3 kg phosphorus, 25 kg potassium and 1.2 kg sulphur besides, organic carbon. Generally, crop residues of different crops contain 80% of nitrogen (N), 25% of phosphorus (P), 50% of sulphur (S) and 20% of potassium (K). If the crop residue is incorporated or retained in the soil itself, it gets enriched, particularly with organic C and N [3]. The states in the North-Western regions of India are facing intense air pollution, particularly during the October–December months, which is the main agricultural harvest season in the country. During these months, the smoke plume emitted from the burning of rice paddy stubble in the open travels across the entire northwest region causing high PM concentrations and dense haze throughout the National Capital Region (NCR)1 of New Delhi [4]. The on-farm burning of crop residues has intensified in recent years due to unavailability of low cost and easily adaptable technologies for its handling and management and short time window availability between harvesting and sowing of crops. In an era of intensive cropping, the disposal of large quantities of crop residues has become menace. Inadequate organic waste management leads to a plethora of problems such as environmental pollution, eutrophication, and aesthetic damage to urban landscape, greenhouse gases emission and effects on human health. Unwise and non-scientific disposal of wastes not only poses a grave threat to environmental quality but also results in loss of economic value of wastes. Since organic wastes are an abundant pool of organic matter and valuable plant nutrients, agricultural recycling of these wastes appears to be a promising alternative enabling value addition and their resourceful utilization [5]. *In-situ* and *ex-situ* management of crop residues have remained a major challenge due to lack of easily adaptable and affordable mechanical and microbiological technologies at farmers' field. Cellulose is recognized as the most abundant biopolymer on earth, which is the major component of agricultural residues [6]. The biopolymer can be degraded into glucose by cellulases such as endoglucanase, cellobiohydrolase and β-glucosidase [7]. Enzymatic degradation of cellulosic biomass is important for sustainable utilization of agricultural residues. Therefore, production of high efficient and economic cellulolytic enzymes, including cellulase, xylanase, and glycosyl hydrolases, has received a great attention. Some important microbes from different niches have been identified to have an extracellular enzyme system to hydrolyze cellulose for metabolism requirement [8]. Chen et al. 2020 reported that *Fictibacillus* sp YS-26 can efficiently degrade different lignocellulosic agricultural residues by producing cellulolytic enzymes viz., *α*-amylase, pectinase, protease and xylanase [9]. Cellulolytic fungi like *Aspergillus awamori*, *Aspergillus nidulans*, *Trichoderma viride* and *Phanerochaete chrysosporium* are recommended for recycling lignocellulosic-rich organic matter and to convert it into nutrient rich compost. However, these inoculants need to be generated on commercial scale for handling large volume of biomass. There are two major methods of inoculum production-solid state and liquid state fermentation. Both liquid and solid-state fermentations have some disadvantages like odour, slow process, and storage and transport problem especially when larger quantity is required. Viability and shelf life of spores are other issues associated with the current bio-inoculum production techniques. Also, the microbial formulation should also be capable of degrading diverse type of agri-residues. Hence, a complete knowledge of the mass production of microbial inoculants and proper delivery system is a critical impediment to microbial products. It is important to consider that the formulation should have shelf life with retained biological activity for up to a year preferably at ambient temperatures, which is a challenging task as it requires greater effort in terms of funding and research[10]. Therefore, it is essential to develop a high throughput protocol that

employs common and cost-effective growth medium, should be easy to operate and consumes less time for mass production of fungal spores. In view of this, the present work was carried out to produce spores in mass of potential fungal strains which can be used for degradation of various agri-residues like paddy straw, garden waste, fruit waste and convert them to valuable product i.e., compost and to monitor the chemical and biological changes during composting.

2. Materials and Methods

2.1 Selection of Fungal Strains: Seven fungi, selected on the basis of their lignocellulolytic potential, namely *Aspergillus awamori (F-18), Aspergillus nidulans, Trichoderma viride, Trichoderma longibrachitum, Drechslera halodes, Eupenicillium crustaceum* and *Paecilomyces variotii* were employed in the present study. *Aspergillus awamori* (F-18), *Aspergillus nidulans* and *Trichoderma viride* were obtained from Division of Microbiology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi while other fungi were isolated, characterized under present investigation. All isolates were maintained on Potato dextrose agar medium and later cultivated in modified jaggery medium [11].

2.2 Production of Fungal Spores: The mass production of spores was carried out on sorghum grains using solid state fermentation. In this procedure, 500g of sorghum grains were boiled, air dried and then coated with 4% calcium carbonate and 2% calcium sulphate. The coated grains were sterilized in autoclave bags at 15 lbs pressure and at a temperature of 121°C for 1 hour. The sterilized bags were cooled to room temperature and inoculated with selected fungi individually using their spores. The inoculated bags were incubated at 30°C for 10 days. After 10 days, the bags were sieved on a sieve machine having different mesh size from 0.5 mm-2.0 mm and spores were collected in small plastic bottles.

2.3 Study of Characteristic Features of Selected Fungal Strains: The different fungal strains were characterized based on shelf life, functional activity as following:

i) Shelf life: Four different additive agents *viz.* vegetable oil, glycerol, tween-80 and paraffin oil were used for study of shelf life of fungal spores. Mixture of spore powder (1g) was incubated at room temperature for shelf-life evaluation. Samples were taken out periodically and plate count method was used for the viability of spores.

ii) Functional activity: The functional activity of spores was evaluated by inoculating 500 mg spores in 5 liters of modified jaggery medium and incubated for 4 days. Fungal mat was mixed uniformly and used as compost inoculum for rapid degradation of substrates. Fungal mat of all the seven fungi was mixed in equal proportion to make consortium for degradation of various agro and horticultural residues using pit method of composting. An experiment was laid in cemented pits of Division of Microbiology, ICAR-IARI, New Delhi using four different substrates namely maize cobs and stalks, paddy straw, kinnow fruit waste and flower waste for rapid degradation. Two cemented pits (1 x 2 x 8 m), i.e., one for test and other as control, for each substrate were filled with 100 kg of selected substrate followed by spraying and thorough mixing of liquid inoculum with the dose of 5 litres per tonne. Throughout the experiment, moisture was maintained at 80% and turning of the material was done periodically after 15, 30 and 60 days.

2.4 Study of Physico-chemical and Microbiological changes during degradation process: During the degradation of different substrates, different hydrolytic enzymes being the mediators of degradative processes, play an important role in degradation [12] and therefore, the changes in activities of enzymes, *viz*., cellulose, xylanase and pectinase were studied

as these are responsible for the hydrolysis of cellulose, hemicellulose and pectins. The samples were collected periodically and analysed for physico-chemical and microbiological parameters *viz.* cellulase and xyalanase [13], pectinase [14], organic carbon [9], total N by Kjeldahl method [15]. In addition, humic substances were also determined [16] and standard procedure was used for the enumeration of microorganisms. The total microbial populations of bacteria, fungi and actinobacteria were determined.

3. Results and Discussion

Sporulation Efficiency: Different fungi were observed to have varying sporulation ability (Table 1). It was observed that the fungal spore count after 10 days of inoculation was higher than the initial stage. Fold increase in sporulation varied from 110 to 118 and the maximum increase in spore count was observed with *Paecilomyces variotii* followed by *Eupenicillium crustaceum* and *Drechslera halodes.* In general, it was observed that all the selected fungal strains were able to produce spores in sufficient quantity as required for rapid degradation of various agro-residues. On the fourth day in surface culture inoculation, *Aspergillus awamorii* showed a spore formation of 5.70± 0.20 x 10^8 per ml of suspension [2]. Another study conducted by [14] showed that *Trichoderma viride* yielded 3 x 10^{18} spores per ml of suspension on paddy as a substrate at the seventh day of inoculation.

Shelf life of spores: The spores of each fungal isolate were evaluated for shelf-life in four additives with dry powder of spores as control at room temperature for 150 days. The spore count of *Aspergillus awamori, Trichoderma viride, Trichoderma longibrachiatum, Drechslera halodes, Eupenicillium crustaceum* and *Paecilomyces variotii* showed that glycerol was found to be the most suitable additive among all the four additives and showed maximum count of spores in 150 days and hence longer shelf-life of spores. The effectiveness of microbial inoculants mainly depends on the type of formulation and the delivery technology which can increase the shelf lives for few months to year [10]. The spore count for all fungal strains remained stable in glycerol except for *Eupenicillium crustaceum* which showed a marginal decrease in spore count after 150 days of storage (Fig. 1). Similar results were obtained by [16] that showed glycerol as an additive at 3% and 6% can increase the shelf life of *Trichoderma* sp. from 4-5 months to 7 months. Vegetable oil was not found suitable additive as the spore count of all the fungal strains

Table 1: Mass production of spores on sorghum grains

S. no	Fungal strains	Spore count		Fold efficiency
		Initial (10^9)	Final (10^{12})	
1	*Aspergillus awamori*	3.0	3.3	110
2	*Aspergillus nidulans*	1.5	2.1	140
3	*Trichoderma viride*	2.4	2.6	108
4	*Trichoderma longibrachiatum*	2.0	3.4	170
5	*Dechslerahalodes*	2.2	2.9	132
6	*Eupenicillium crustaceum*	1.3	1.8	138
7	*Paecilomyces variotii*	1.6	3.0	188
	C.D. (@5 %)	**0.24**	**0.30**	**-**

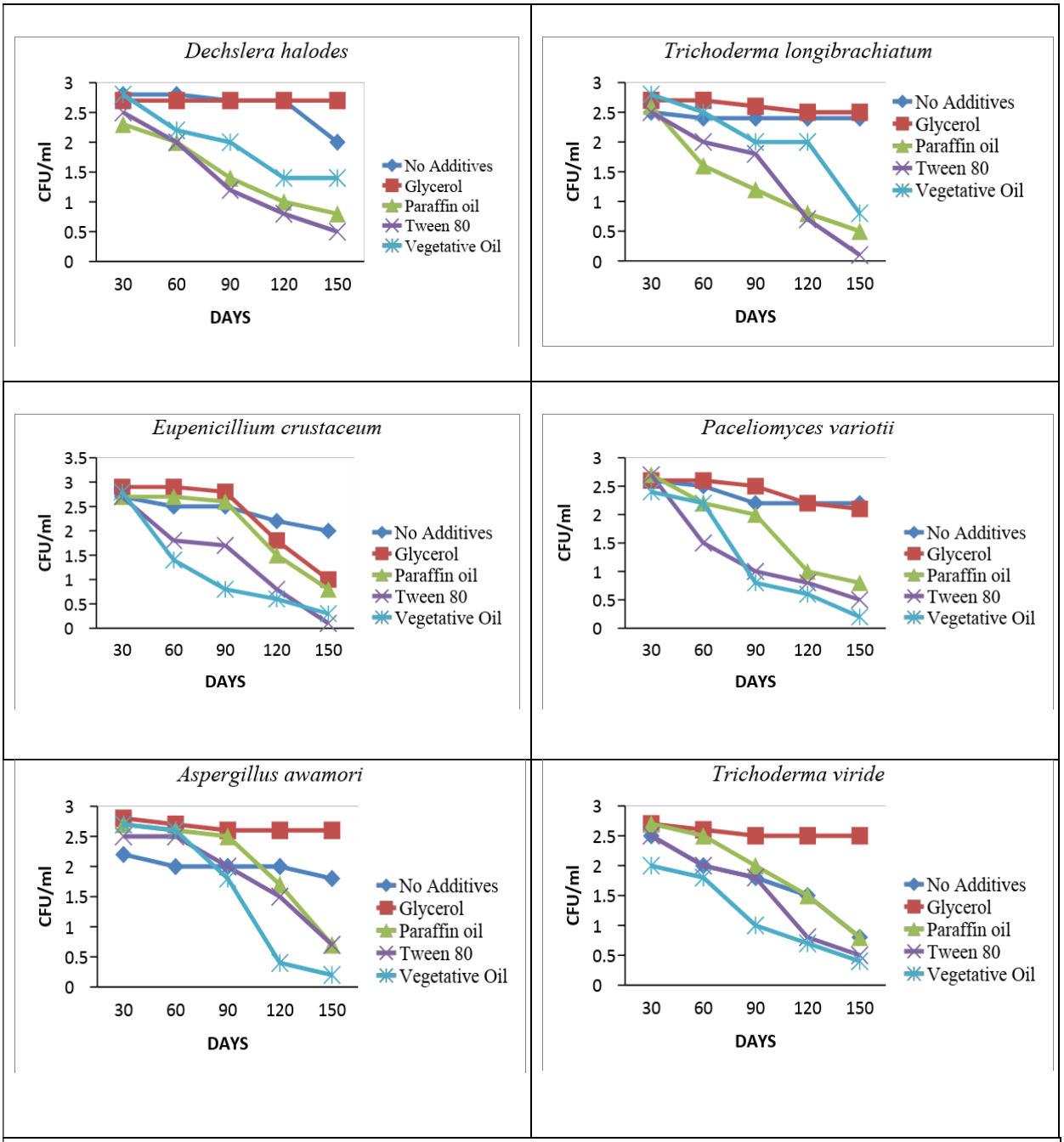

Fig. 1 Shelf Life of Fungal Spores in Various Additives

drastically decreased after 30 days of storage. The spore count in paraffin oil varied with fungal strains and shelf- life ranged from 60-90 days only. The spore powder stored at room temperature for all the fungal strains also showed stable count in 120 days. Hence, the present study was carried out keeping in view the criteria of low production cost and ease in handling while developing a microbial formulation.

Microbial population dynamics during degradation: The microbial population at various stages of degradation was also determined (Table 2a & 2b). The bacterial and

actinobacterial population were found to increase upto 30 days and then declined, while fungal population first increased upto 45 days and then it was found to be stable. The highest bacterial and actinobacterial populations were observed in flower waste and kinnow waste, respectively, whereas fungal population was almost same in all the treated wastes. The study carried out by [17] suggested that the number of cellulolytic microorganisms was constantly and significantly higher in the treatment with fungal inoculation, depicting that the initial inoculation significantly enhanced the population density of cellulolytic microorganisms. On the other hand, in the same study, during the thermophilic stage, at the time of start no significant effect on population density of cellulolytic microorganisms, depicting that the added ligno-cellulolytic fungi were partially killed or inactivated during the thermophilic stage. Fungi as reflected by their population were found to be involved in degradation process. The succession of microbes plays a key role in degradation of substrates and converting to compost and the presence or appearance of some microorganisms also reflect the quality of maturing**.** In the present study dilution plating technique was used which clearly showed variation in microbial population with various substrates.During the degradation of maize, paddy straw, kinnow and flower waste, the cellulose activity increased at 30 days in the fungal consortium applied treatments and it declined after 60 days in maize and paddy straw while in kinnow and flower degradation complete decline was observed by 45 days (Fig. 2 a). However, in a degradation study conducted by [15], *Aspergillus niger* was reported to produce cellulase when incubated with paddy straw maximally at 48 hours and minimum at 24 hours. The initial xylanase activity in all the substrates showed an increase after 15 days and the maximum activity increased upto 60 days in case of inoculated paddy straw and maize waste and declined later on (Fig. 2 b). The xylanase enzyme produced by *Aspergillus* sp. is also found to be efficient in pup bleaching [15]. This observation

Table 2(a) Changes in microbial population during composting of various waste

Treatments	**Bacteria (X 10^8cfu g^{-1} soil)**				**Fungi (X 10^3cfu g^{-1} soil)**				**Actinobacteria (X 10^4cfu g^{-1} soil)**			
	Days				Days				Days			
	0	15	30	45	0	15	30	45	0	15	30	45
MCS	229	235	278	269	10	15	18	07	36	169	190	160
MCS+ MC	231	257	264	271	11	26	32	22	43	174	203	172
FW:DL(1:1)	241	264	309	336	08	11	13	10	30	156	106	221
FW:DL(1:1) + MC	226	284	321	199	10	27	31	22	28	134	184	231
KW:DL (1:1)	201	291	334	241	12	18	22	18	34	148	203	162
KW:DL(1:1)+ MC	**225**	**269**	**321**	**195**	**12**	**26**	**30**	**20**	**21**	**114**	**221**	**211**

Table 2(b) Changes in microbial population during composting of Paddy straw

Treatments	**Bacteria (X 10^8cfu g^{-1} soil)**					**Fungi(X 10^3cfu g^{-1} soil)**					**Actinomycetes(X 10^4cfug^{-1}soil)**				
	Days					Days					Days				
	0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
PS	216	236	248	250	253	11	16	19	20	22	20	152	189	206	135
PS+ MC	**223**	**251**	**279**	**291**	**223**	**10**	**23**	**29**	**35**	**30**	**24**	**168**	**199**	**221**	**153**

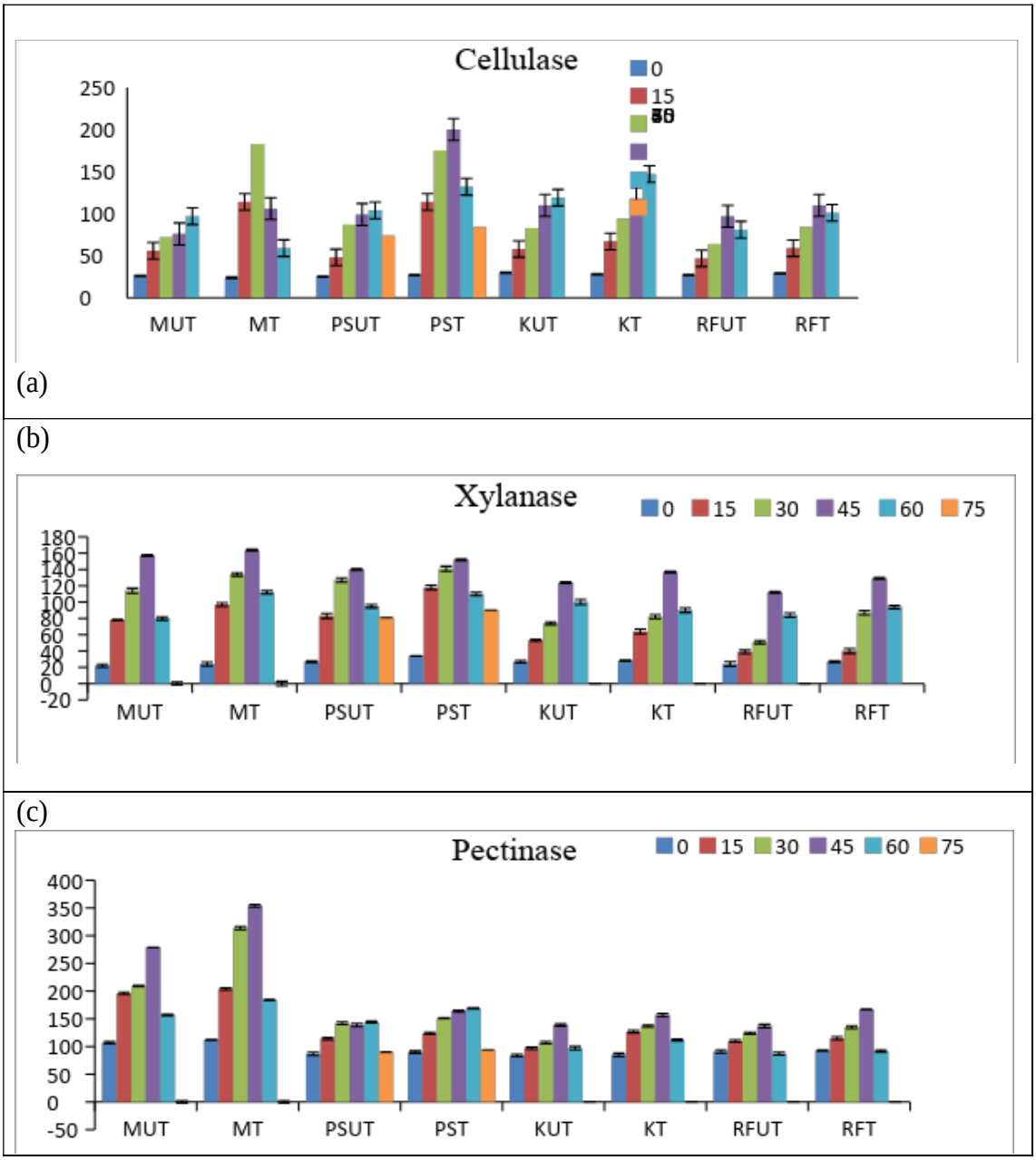

Fig. 2 Enzymatic activity of waste during degradation (mg reducing sugar kg -1dry matter h-1)

MUT: Maize Untreated	***MT: Maize treated***	***PSUT: Paddy Straw Untreated***
PST: Paddy Straw Treated	***KUT: Kinnow Untreated***	***KT: Kinnow Treated***
RFUT: Flower Untreated	***RFT: Flower Treated***	

on cellulase and xylanase activity shows that cellulose and hemicellulose content of the substrates are degraded using fungal consortium. During composting of various substrates, pectinase activity was also found to vary with time (Fig. 2 c). The research work done on cellulase, protease activities in composts prepared using pig slurry, horse manure and straw showed

Table 3: Changes in C:N Ratio during degradation in Pits

	Days						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Maize Untreated	51.00	41.00	39.00	32.00	28.00	-	22.00
Maize Treated	52.00	40.00	38.00	29.00	27.00	-	20.00
Paddy straw Untreated	50.00	40.00	32.00	30.00	28.00	26.00	25.00
Paddy straw Treated	52.00	41.00	30.00	29.00	25.00	22.00	22.00
Flower Untreated	48.00	41.00	38.00	37.00	-	-	-
Flower Treated	49.00	38.00	35.00	25.00	-	-	-
Kinnow Untreated	42.00	39.00	31.00	29.00	-	-	-
Kinnow Treated	**41.00**	**37.00**	**30.00**	**25.00**	-	-	-

lower enzyme activities than the present work. A study by [18], after assessing 16 compost quality parameters for seven different substrates at five different stages, confirmed that the consortia of fungal inoculants *viz*., *Trichoderma viride, Rhizomucor pussillus, Aspergillus awamori* and *Aspergillus flavus* are able to efficiently decompose all the crop residues. The changes in total organic carbon content were also determined during degradation of various substrates. The initial organic carbon of substrates varied from 40.9 % to 49.8%. As the decomposition progressed, the organic carbon content decreased and at the last stage of composting, the lowest organic carbon content was observed with maize waste after 60 days followed by flower waste after 45 days. Similar results were reported by [12] on organic carbon mineralization in paddy straw degradation, where 55% of organic carbon mineralization took place at a temperature of 27^{o}C in 160 days. It was also observed that inoculated organic waste degraded faster than un-inoculated wastes. In the present study, at the initial stages, the total N content of different wastes varied from 0.7 % to 1.0%, where the maximum was found in paddy straw followed by maize waste, flower waste and kinnow waste. As the degradation process progressed, the total N also increased with time and varied from 1.1% to 1.5%. Similarly, the initial C:N ratio varied in all the substrates, paddy straw and maize had high C:N ratio as compared to flower waste and Kinnow waste. Flower and kinnow waste degradation was completed in 45 days while in maize and paddy straw, the same were degraded in75 days and 90 days, respectively. Thus, to reach the ideal C: N ratio of 20:1, the time of decomposition varied with substrates (Table 3). Although, C:N ratio is one of the parameters of maturity of compost, the stability of compost is also very important factor and the extent of organic matter stabilized is also helpful. The finished product was analyzed for phytotoxicity, humus%, pH, EC, S% and K% (Fig. 3).

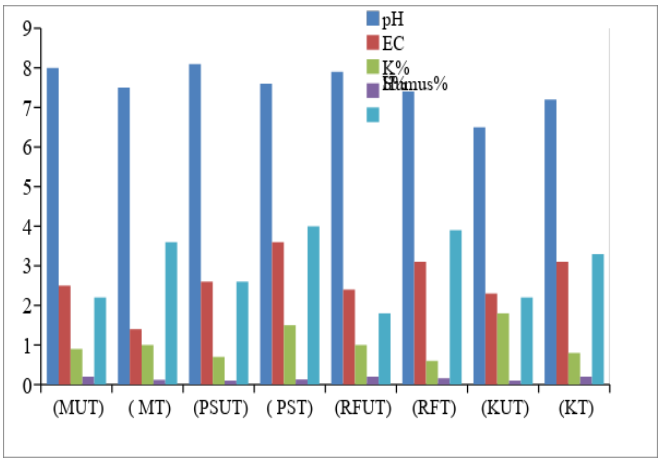

Fig.3 Changes in the physiological properties during the degradation of various waste

MUT: Maize Untreated; MT: Maize treated; PSUT: Paddy Straw Untreated; PST: Paddy Straw Treated; KUT: Kinnow Untreated; KT: Kinnow Treated;RFUT: Flower Untreated; RFT: Flower Treated

The results clearly showed variation in most of the parameters with substrates used for composting. The pH range was 6.5-8.0 in all the

substrates but in inoculated substrates the range of pH was 7.2-7.6 while EC was 1.4-3.6 and the humus content also varied with substrates. The highest humus% was found in paddy straw followed by maize, flower and kinnow. The final product also showed variation in K% and S% which were found to be 0.6% -1.8% and 0.1% - 0.20%, respectively. Seed germination test was performed with water cress seeds (*Lepidium sativum*) and it was observed that all the substrates where fungal inoculation was carried out showed 100% seed germination while the uninoculated substrates showed 50% - 60% seed germination clearly indicating that the compost had not matured.

Overall results on spore count of *Aspergillus awamori, Trichoderma viride, Trichoderma longibrachiatum, Dechslera halodes, Eupenicillium crustaceum* and *Paecilomyces variotii* showed that glycerol was the most suitable additive for incubation and longer shelf-life of fungal spores. The fungal strains *Trichoderma viride, Trichoderma longibrachitum, Dechslera halodes, Eupenicillium crustaceum* and *Paecilomyces variotii* were found effective in degradation of ligno-cellulolytic material and hence can be used for degradation of different agro-wastes.

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Compliance with ethical standards: The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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