

Evaluation of different microbial consortia for decomposing different wastes in *in vitro* and *in vivo*

ABSTRACT

If not managed properly, agricultural solid waste can cause environmental and health problems, including pollution and respiratory issues. Recycling these wastes through composting is a viable method to enhance soil health, reduce environmental impact and mitigate climate change. The results demonstrated that microbial consortia, particularly the T₇ consortium (*T. h.* + *T. k.* + *P. c.* + *A. f.* + *A. n.* + *B. s.* + *P. f.*), significantly accelerated the composting process, reducing decomposition time and improving compost maturity compared to single microbial cultures and un-inoculated controls. Efficient composting helps in waste management and improves soil quality and sustainable Agriculture. Key observations over a 105-day composting period included changes in texture, total days required, moisture content and pH. Microbial treatments also led to favorable changes in moisture retention, pH stabilization towards neutral or slightly alkaline conditions, and enhanced nutrient content, particularly with *Trichoderma harzianum* and *Pseudomonas fluorescens*. The study reveals that the use of efficient microbial consortia can substantially enhance the composting of agricultural residues, contributing to effective waste management and sustainable agricultural practices.

(**Keywords:** Biodegradation, consortia, agriculture waste management, microbial consortia.)

INTRODUCTION

Agriculture is one of the main sectors that generates materials for the bio-economy. The organic wastes production has increased with rapid increase in population, agriculture production and industrialization development. These wastes are causing a threat to the environment by releasing pollutants and toxic gases (Zhang *et al.*, 2013). Recycling of agricultural wastes by composting is a viable approach for a healthy environment. These materials can be recycled and reused to replenish soil carbon, crop nutrition and mitigate climate change (Abro, *et al.*, 2017). Soil microbes play a central role in the decomposition of organic residue in soils, and the rate of this turnover can be increased by microbial enhancement using microbially enhanced compost extracts.

Utilizing the micro-organism *viz. Trichoderma* sp., the crop residues like cane trash, paddy trash, wheat trash and press mud cake can be recycled into good quality compost not only at pit level but in situ also, which will improve organic matter along with macro, micronutrients, physico-chemical and biological conditions of soil (Sharma, *et al.*, 2012). The recycling of agricultural wastes can bring tremendous benefits to agriculture and land management in a long run. Additionally, there are the benefits of a cleaner environment, a healthier habitat and an intelligent use of all available recyclable resources without labelling them as wastes. The objectives of composting are to stabilize the putrescible organic matter in raw agricultural wastes to reduce the offensive odour, to kill weed seeds, pathogenic organisms and finally to produce a uniform, slow release organic fertilizer which stimulates soil life, improves soil structure, helps plants to resist pests and diseases.

MATERIALS AND METHODS

2.1. Collection of different agricultural bio-wastes

Different bio-wastes used for decomposition were collected from the located area from an adjoining farmer's field and brought to the laboratory to conduct series of experiments. Bio-wastes such as Wheat straw, Soybean waste, Sugarcane bagasse, Banana tree waste and Fruit waste were used in the experiment.

2.2 Procurement of microbial culture

Decomposing culture like *T. harzianum*, *T. koningii*, *P. fluorescence*, *B. subtilis*, *A. niger*, *A. flavus*, and *P. chrysogenum* was obtained from different sources like farmer's pit area, decomposing site, forest dump area, *etc.* The fungi were isolated successfully by serial dilution on Potato Dextrose Agar plate and bacteria on Nutrient Agar plate. The pure culture of fungus as well as bacteria was maintained on PDA and NA media respectively.

2.2. Preparation of liquid microbial culture

For fungus, PDA broth was prepared, and five disks of different fungal organisms was added in that and kept for multiplication. After some days the growth of fungus was came on the liquid broth in the form of mat. The fully grown up mat was taken out from flask, then that mat was mixed by using electronic mixture to make it homogenous and then mixed in distilled water to make liquid microbial culture for taking treatments. For bacteria, the inoculum was added in NA broth and kept for multiplication. After some days there was scattered growth of bacteria in broth. These broths were then mixed

homogenously and used for treatments. By mixing these different decomposing liquid cultures in same proportion and treatments are taken with three different concentrations (Islam et al., 2014). The process was conducted under laminar air flow.

2.3. *In vitro* evaluation of consortia of microbial decomposers for decomposing different wastes

For *in vitro* experiment, different liquid microbial cultures were taken and mixed with same proportion to prepare spore solution of seven different consortia. Different concentrations (5ml, 10ml, 15ml) were taken and prepared seven consortia were added to each polythene bag (having a thickness of 51 microns and above) containing 50 g SOW (Solid Organic Wastes) each at anaerobic condition. Moisture percentage was maintained at > 60 %. This experiment was conducted under laminar air flow.

2.4. *In vivo* evaluation of consortia of microbial decomposers for decomposing different wastes

For *in vivo* experiment different liquid microbial cultures were taken and mixed with same proportion to prepare spore solution. Different concentrations were taken and prepared seven consortia were added to each polythene bags (having thickness of 51 microns and above) containing 1kg SOW (Solid Organic Wastes) each aerobic condition. The entire experiment was replicated three times. Moisture percentage was maintained at > 60 %. This experiment was conducted under field condition.

2.6. Quality compost parameter

2.6.1. Texture of decomposed matter

Texture of decomposed matter of different straw was observed after 105 Days After Inoculation of different micro-organisms.

2.6.2. Total days required for decomposition

Total days required for decompositions were recorded on the basis of quality compost parameters.

2.6.3. Moisture content estimation

Samples were taken in a filter paper and their initial weights were recorded. Afterwards, the samples were dried in hot air oven. Moisture content in compost was recorded after maturation period by gravimetric method (FCO, 1985).

2.6.3. pH

Samples were taken in 100 ml beaker and diluted 1:10 (1 part sample in 10 parts of distilled water) and placed on shaker for 1 hr. The samples were centrifuged at 4000

rpm for 30 min. and filtered through Whatman No.1 filter paper. pH of the suspension was measured potentiometrically using a combined glass electrode. (Gat, 2020).

2.7. Statistical analysis

The data pertaining, today's required for composting, moisture content, pH of compost obtained during the present investigation were processed and tested for statistical significance by using Analysis of Variance (ANOVA) and also Statistical analysis books referred which were written by Panse and Sukhatme as well as Gomez and Gomez. For statistical analysis, OPSTAT was utilised online and also concerned analysis work done in formulated excel sheets too.

RESULTS AND DISCUSSION

Tables 1, 2, 3 and 4 show the characteristics of SOW in *in vitro* and *in vivo* bioconversion respectively using different fungal and bacterial decomposing cultures.

3.1. Texture of decomposed matter

The data regarding texture presented in table no.1 represents texture of different substrates due to different consortia. Texture of decomposed matter of different straw was observed after 105 DAI. The differences in texture of compost after maturity during *in vitro* and *in vivo* composting due to application of different microbial consortia was observed in different substrates like wheat straw, soybean straw, sugarcane bagasse, banana tree waste and fruit waste. Mostly smooth, very smooth, rough and rather rough texture of decomposed matter were observed by different substrates after maturity of compost.

3.2. Total days required for decomposition

The data regarding number of days for decomposition presented in table no.2. Results of present study revealed that total days for decomposition of Wheat straw, soybean straw, sugarcane bagasse, banana tree waste, and fruit waste were decreased due to application of decomposing cultures than untreated control. The reduction in time over control ranged from 28 to 47 % in different substrates. Fruit waste decomposed at the

Table 1: Texture of decomposed matter for *in vitro* and *in vivo* bioconversion of different bio-waste into compost by the application of different decomposing consortia

Tr. No	Treatments	Texture of decomposed matter									
		<i>In vitro</i>					<i>In vivo</i>				
		Wheat straw	Soybean straw	Sugarcane bagasse	Banana tree waste	Fruit waste	Wheat straw	Soybean straw	Sugarcane bagasse	Banana tree waste	Fruit waste
T ₁	<i>T. h. + T. k.</i>	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
T ₂	<i>B. s. + P. f.</i>	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
T ₃	<i>A. f. + A. n. + P. c.</i>	Smooth	Smooth	Rather smooth	Very smooth	Very smooth	Smooth	Smooth	Smooth	Very smooth	Smooth
T ₄	<i>T. h. + T. k. + P. c.</i>	Smooth	Smooth	Rather smooth	Very smooth	Smooth	Smooth	Smooth	Rather smooth	Very smooth	Smooth
T ₅	<i>B. s. + P. f. + T. h. + T. k.</i>	Smooth	Smooth	Rather smooth	Very smooth	Rather smooth	Smooth	Smooth	Rather smooth	Very smooth	Smooth
T ₆	<i>T. h. + T. k. + A. f. + A. n.</i>	Smooth	Smooth	Rough	Very smooth	Rough	Smooth	Smooth	Rough	Very smooth	Smooth
T ₇	<i>T. h. + T. k. + P. c. + A. f. + A. n. + B. s. + P. f.</i>	Smooth	Smooth	Rough	Very smooth	Rough	Smooth	Smooth	Rough	Very smooth	Rough
T ₈	Control (Untreated)	Rather Rough	Rather Rough	Rather Rough	Rather Rough	Rather Rough	Rather Rough	Rather Rough	Rather Rough	Rather Rough	Rather Rough

Table 2: Days required for decomposition for *in vitro* and *in vivo* bioconversion of different bio-waste into compost by the application of different decomposing consortia

Tr. No	Treatments	Days required for decomposition									
		<i>In vitro</i>					<i>In vivo</i>				
		Wheat straw	Soybean straw	Sugarcane bagasse	Banana tree waste	Fruit waste	Wheat straw	Soybean straw	Sugarcane bagasse	Banana tree waste	Fruit waste
T ₁	<i>T. h. + T. k.</i>	75	78	81	69	48	74	72	65	67	46
T ₂	<i>B. s. + P. f.</i>	76	78	83	71	49	76	74	66	69	46
T ₃	<i>A. f. + A. n. + P. c.</i>	71	71	80	60	47	69	66	63	59	44
T ₄	<i>T. h. + T. k. + P. c.</i>	69	74	78	60	48	67	69	61	59	45
T ₅	<i>B. s. + P. f. + T. h. + T. k.</i>	70	78	78	65	48	68	73	61	63	46
T ₆	<i>T. h. + T. k. + A. f. + A. n.</i>	62	70	71	63	45	62	61	58	56	43
T ₇	<i>T. h. + T. k. + P. c. + A. f. + A. n. + B. s. + P. f.</i>	66	68	74	74	46	63	65	60	57	44
T ₈	Control (Untreated)	118	105	104	93	80	117	103	103	91	78

Table 3: Moisture per cent of decomposed matter for *in vitro* and *in vivo* bioconversion of different bio-waste into compost by the application of different decomposing consortia

Sr. No.	Treatments	Moisture per cent									
		<i>In vitro</i>					<i>In vivo</i>				
		Wheat straw	Soybean straw	Sugarcane bagasse	Banana tree waste	Fruit waste	Wheat straw	Soybean straw	Sugarcane bagasse	Banana tree waste	Fruit waste
T ₁	<i>T. h. + T. k.</i>	31.83	33.0	29.16	32.00	35.3	26.00	27.16	26.33	27.00	26.5
T ₂	<i>B. s.+ P. f.</i>	36.00	36.6	31.5	37.0	37	30.16	28.00	30.5	28.16	30.16
T ₃	<i>A. f. + A. n.+ P. c.</i>	33.6	34.3	30.33	34.3	35.0	26.5	29.1	26.5	28.8	26.83
T ₄	<i>T. h. + T. k + P. c.</i>	35.00	36.0	31.6	35.3	37.83	23.83	26.16	24.00	26.00	23.8
T ₅	<i>B. s. + P. f.+ T. h. + T. k.</i>	30.3	31.33	30.3	30.3	32.8	25.00	25.3	25.16	25.33	25.33
T ₆	<i>T. h. + T. k + A. f. + A. n</i>	32.3	33.6	31.3	33.0	34.5	25.00	26.1	25.83	26.3	26.3
T ₇	<i>T. h. + T. k + P. c+ A. f. + A. n.+ B. s. + P. f</i>	34.33	35.0	34.33	34.3	35.9	28.00	26.8	28.16	27.16	27.66
T ₈	Control (Untreated)	35.6	36.6	35.6	36.0	38.5	26.83	26.83	27.00	26.3	27.16
	S.E.(m) ±	0.80	1.06	1.19	0.76	1.01	0.63	0.67	0.76	0.60	0.77
	C.D. (P=0.01 & 0.05)	3.3	4.4	4.9	3.16	4.2	2.61	2.80	3.16	2.52	3.19

Table 4: Change in pH of decomposed matter for *in vitro* and *in vivo* bioconversion of different bio-waste into compost by the application of different decomposing consortia

Sr. No.	Treatments	Change in pH (mean)																			
		<i>In vitro</i>										<i>In vivo</i>									
		Wheat straw		Soybean straw		Sugarcane bagasse		Banana tree waste		Fruit waste		Wheat straw		Soybean straw		Sugarcane bagasse		Banana tree waste		Fruit waste	
		Initial	Final	Initial	Final*	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T ₁	<i>T. h. + T. k.</i>	7.20	7.10	7.16	7.1	7.12	7.12	7.15	7.11	7.2	7.25										
T ₂	<i>B. s.+ P. f.</i>	7.22	7.10	7.18	7.3	7.10	7.14	7.21	7.23	7.3	7.11										
T ₃	<i>A. f. + A. n.+ P. c.</i>	7.16	7.20	7.1	7.21	7.21	7.1	7.11	7.3	7.21	7.16										
T ₄	<i>T. h. + T. k + P. c.</i>	7.12	7.22	7.3	7.11	7.11	7.1	7.21	7.13	7.1	7.31										
T ₅	<i>B. s. + P. f.+ T. h. + T. k.</i>	7.08	7.31	7.0	7.3	7.31	7.12	7.16	7.11	7.08	7.24										
T ₆	<i>T. h. + T. k + A. f. + A. n</i>	7.07	7.10	7.0	7.12	7.1	7.01	7.12	7.14	7.06	7.14										
T ₇	<i>T. h. + T. k + P. c+ A. f. + A. n.+ B. s. + P. f</i>	7.06	7.00	7.0	7.1	7.01	7.06	7.03	7.14	7.01	7.08										
T ₈	Control (Untreated)	7.71	6.7	7.1	6.4	6.6	7.7	7.8	7.5	7.4	7.64										

fastest rate while sugarcane bagasse required maximum time for decomposition in all the treatments. The differences in average number of days required for compost maturity during *in-vitro* and *in vivo* composting due to application of different microbial consortia were found statistically significant. The decomposing cultures were found significantly superior in reducing the decomposition time of substrates over un-inoculated control. These results are in conformity with Game (2015), reported that Test consortium reduced the composting period of rural and urban waste by 22.68% and 18.39%, respectively over uninoculated control.

3.3. Moisture content

The data presented in Table no. 3 shows moisture content of composts from different substrates taken. Results of present study revealed that moisture content of wheat straw, soybean straw, sugarcane bagasse, banana tree waste, and fruit waste were recorded different at compost maturity. The moisture content of all substrates generally shifted toward 24% to 35%. Maximum moisture content in wheat straw was recorded by consortium T₂(36 %) and minimum by consortium T₂(26 %). In soybean straw maximum moisture content was recorded by consortium T₂(36.6 %) and minimum by consortium T₅(25.3 %). In sugarcane bagasse maximum moisture content was recorded by consortium T₇(34.33 %) and minimum by consortium T₄(24 %). Similar discussions and results are in conformity with Dadas (2003). He reported that minimum moisture content ie. 24.5 % was found in compost prepared by sugarcane trash and maximum by bajra stubbles ie. 26.3%.

3.4. pH

Results of present study revealed that pH of wheat straw, soybean straw, sugarcane bagasse, banana tree waste, and fruit waste were decreased and tend towards neutral value both in *in vitro* and *in vivo*. The pH of all substrates generally shifted toward neutral or slightly alkaline at maturity. The highest pH was observed in the un-inoculated soybean straw (7.8), and the lowest in un-inoculated banana tree waste (6.4). These above mentioned results are in conformity with Nielsen *et al.*, (1997); Ranali *et al.*, (2001); De Olivera *et al.*, (2002); Adebayo *et al.*, (2011), Himanen&Hanninen, (2011)and Gat, (2020), Goyal *et al.*, (2020).

CONCLUSIONS

The microbial treatments, particularly consortia like T₆ (*T. h.* + *T. k.* + *A. f.* + *A. n.*) and T₇ (*T. h.* + *T. k.* + *P. c.* + *A. f.* + *A. n.* + *B. s.* + *P. f.*) significantly enhanced the composting process. These treatments accelerated decomposition, improved compost quality by optimizing texture, pH, moisture content, effectively reduced composting time and enhancing the quality of the final product both in field and lab conditions. The study reveals that the use of efficient microbial consortia in *in vivo* than *in vitro* can substantially enhance the composting of agricultural residues, contributing to effective waste management and

sustainable agricultural practices. Efficient composting using selected microbial cultures can mitigate the environmental and health issues associated with agricultural solid waste by transforming it into valuable organic fertilizer. These consortia offer a promising solution for faster and more effective waste management, contributing to both environmental sustainability and agricultural productivity. The consortia T₇ and T₆ will be effectively used for faster decomposition of organic wastes.

Overall, microbial consortia outperformed individual cultures, making them a promising solution for sustainable agricultural waste management.

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Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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