

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

ABSTRACT

Agricultural solid waste, if not managed properly, 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, significantly accelerated the composting process, reducing decomposition time and improving compost maturity compared to single microbial cultures and un-inoculated controls. Efficient composting not only helps in wastes management but also improves soil quality and sustainable Agriculture. Key observations over a 105-day composting period included changes in texture, total days 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

The agriculture is one of the main sectors generating the materials for bio-economy. The organic wastes production has increased with rapid increase in population, agriculture production and industrialization development. These wastes are causing threat to environment by releasing pollutants and toxic gases (Zhang *et al.*, 2013). Recycling of agricultural wastes by composting is a viable approach for healthy environment. These materials can be recycled and reutilized to replenish soil carbon, crop nutrition and mitigate climate change (Abro, *et al.*, 2017). Soil microbes play a central role in 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,

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wheat trash and press mud cake can be recycled into good quality compost not only in 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. In addition there are the benefits of a cleaner environment, a healthier habitat and an intelligent use of all available recyclable resources without condemning them as wastes. The objectives in 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.

MATERIAL AND METHODS

2.1. Collection of different agricultural bio-wastes

Different bio-wastes used for decomposition were collected from the located area from adjoining farmer's field and brought to laboratory to conduct series of experiments. Used bio-wastes in experiment were mentioned below,

1. Wheat straw
2. Soybean waste
3. Sugarcane bagasse
4. Banana tree waste
5. Fruit waste

2.2. Decomposing 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.

2.3. Preparation of liquid microbial culture

For fungus, PDA broth was prepared, five disks of different fungus 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 homogeneously and

Comment [NK2]: Rewrite the sentence as: bio-wastes such as (mention 1-5) were used in the experiment.

Comment [NK3]: Change the subheading as: procurement of microbial culture

Comment [NK4]: It would be better if you mention how did the organisms were identified initially for its liquid culture preparation

Comment [NK5]: Fungal organisms

Comment [NK6]: Quote a reference for this methodology (both for fungi and bacteria)

used for treatments. By mixing these liquid cultures in same proportion and treatments are taken with different concentrations.

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

In vitro experiment, different organisms was taken and mixed with same proportion to prepared spore solution. Different concentrations were taken and prepared seven consortia were added to each polythene bag (having thickness 51 micron and above) containing 50 g SOW each. Moisture percentage was maintained at > 60 %. This process was conducted under laminar air flow.

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Comment [NK9]: Mention the different concentrations taken for the exoeriment

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

In vivo experiment different concentrations were taken and mixed with same proportion to prepared spore solution. Different concentrations were taken and prepared seven consortia were added to each polythene bags (having thickness 51 micron and above) containing 1kg SOW each. The entire experiment was replicated three times. Moisture percentage was maintained at > 60 %. This process was conducted under field condition.

Comment [NK12]: Start with for in vivo experiments,

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2.6. Quality compost parameter

2.6.1. Texture of decomposed matter

Texture of decomposed matter of different straw was observed after 105 DA of different micro-organism.

Comment [NK14]: Days after inoculation

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

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).

Comment [NK15]: Moisture content estimation

Comment [NK16]: Reference missing

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.

Comment [NK17]: Quote a reference

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 Sukatme as well as Gomez and Gomez. For statistical analysis, OPSTAT was utilised online and also concerned analysis work done in formulated excel sheets too.

Comment [NK18]: Add reference in the reference section

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.</i> + <i>T. k.</i>	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
T ₂	<i>B. s.</i> + <i>P. f.</i>	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
T ₃	<i>A. f.</i> + <i>A. n.</i> + <i>P. c.</i>	Smooth	Smooth	Rather smooth	Very smooth	Very smooth	Smooth	Smooth	Smooth	Very smooth	Smooth
T ₄	<i>T. h.</i> + <i>T. k.</i> + <i>P. c.</i>	Smooth	Smooth	Rather smooth	Very smooth	Smooth	Smooth	Smooth	Rather smooth	Very smooth	Smooth
T ₅	<i>B. s.</i> + <i>P. f.</i> + <i>T. h.</i> + <i>T. k.</i>	Smooth	Smooth	Rather smooth	Very smooth	Rather smooth	Smooth	Smooth	Rather smooth	Very smooth	Smooth
T ₆	<i>T. h.</i> + <i>T. k.</i> + <i>A. f.</i> + <i>A. n.</i>	Smooth	Smooth	Rough	Very smooth	Rough	Smooth	Smooth	Rough	Very smooth	Smooth
T ₇	<i>T. h.</i> + <i>T. k.</i> + <i>P. c.</i> + <i>A. f.</i> + <i>A. n.</i> + <i>B. s.</i> + <i>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.</i> + <i>T. k.</i>	75	78	81	69	48	74	72	65	67	46
T ₂	<i>B. s.</i> + <i>P. f.</i>	76	78	83	71	49	76	74	66	69	46
T ₃	<i>A. f.</i> + <i>A. n.</i> + <i>P. c.</i>	71	71	80	60	47	69	66	63	59	44
T ₄	<i>T. h.</i> + <i>T. k.</i> + <i>P. c.</i>	69	74	78	60	48	67	69	61	59	45
T ₅	<i>B. s.</i> + <i>P. f.</i> + <i>T. h.</i> + <i>T. k.</i>	70	78	78	65	48	68	73	61	63	46
T ₆	<i>T. h.</i> + <i>T. k.</i> + <i>A. f.</i> + <i>A. n.</i>	62	70	71	63	45	62	61	58	56	43
T ₇	<i>T. h.</i> + <i>T. k.</i> + <i>P. c.</i> + <i>A. f.</i> + <i>A. n.</i> + <i>B. s.</i> + <i>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.</i> + <i>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.</i> + <i>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.</i> + <i>A. n.</i> + <i>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.</i> + <i>T. k.</i> + <i>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.</i> + <i>P. f.</i> + <i>T. h.</i> + <i>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.</i> + <i>T. k.</i> + <i>A. f.</i> + <i>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.</i> + <i>T. k.</i> + <i>P. c.</i> + <i>A. f.</i> + <i>A. n.</i> + <i>B. s.</i> + <i>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.</i> + <i>T. k.</i>	7.20	7.10	7.16	7.1	7.12	7.12	7.12	7.12	7.12	7.12	7.15	7.11	7.2	7.25						
T ₂	<i>B. s.</i> + <i>P. f.</i>	7.22	7.10	7.18	7.3	7.10	7.10	7.14	7.21	7.23	7.11	7.3	7.16	7.16	7.11						
T ₃	<i>A. f.</i> + <i>A. n.</i> + <i>P. c.</i>	7.16	7.20	7.1	7.21	7.21	7.1	7.11	7.3	7.21	7.16	7.1	7.16	7.16	7.16						
T ₄	<i>T. h.</i> + <i>T. k.</i> + <i>P. c.</i>	7.12	7.22	7.3	7.11	7.11	7.11	7.21	7.13	7.31	7.31	7.1	7.16	7.16	7.16						
T ₅	<i>B. s.</i> + <i>P. f.</i> + <i>T. h.</i> + <i>T. k.</i>	7.08	7.31	7.0	7.3	7.31	7.31	7.12	7.16	7.16	7.16	7.11	7.08	7.24							
T ₆	<i>T. h.</i> + <i>T. k.</i> + <i>A. f.</i> + <i>A. n.</i>	7.07	7.10	7.0	7.12	7.12	7.12	7.01	7.12	7.14	7.06	7.14	7.06	7.14							
T ₇	<i>T. h.</i> + <i>T. k.</i> + <i>P. c.</i> + <i>A. f.</i> + <i>A. n.</i> + <i>B. s.</i> + <i>P. f.</i>	7.06	7.00	7.0	7.1	7.01	7.01	7.06	7.03	7.14	7.01	7.14	7.01	7.08							
T ₈	Control (Untreated)	7.71	6.7	7.1	6.4	6.6	6.6	7.7	7.8	7.5	7.4	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).

Comment [NK19]: Mention what results obtained by Game 2015 linking to your results

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).

Comment [NK20]: Mention what results obtained by Dadas 2003 linking to your results

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 Saker *et al.*, (2013).

Comment [NK21]: Reference missing

CONCLUSION

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, and **and**effectively reduced composting time and enhancing the quality of the final product both in field and lab conditions. 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.

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

References

- Abro, S. A., Gadehi, M., Chohan, M., Memon, S. R. & Abro, A. A. (2017). Periodic measurements of CO₂ emission dynamics from wheat straw and its biochar added to soil. *Sindh University Research Journal*, 49(4), 797-800.
- Adebayo, O. S., Kabbashi, N. A., Alam, M. Z., Salihu, A., Abass, A. & Ruqayyah, T. L. D. (2011). Composting of food and yard wastes by locally isolated fungal strains. *African Journal of Biotechnology*. 10(81), 18800-18806.
- Dadas, S. J. (2003). *Studies on preparation of composts from different crop residues and their characterization. (Master's Thesis)*. Mahatma Phule Krishi Vidhyapeeth, Rahuri.
- De Oliveira, S. C., Provenzano, M. R., Satiago Silva, M. R. & Senesi, N. (2002). Maturity degree of composts from municipal solid wastes evaluated by differential scanning calorimetry. *Journal of Environmental Technology*. 23(2), 1099-1105.
- Game, B. C. (2015). *Development of microbial consortium for composting of rural and urban waste (Master's Thesis)*. Mahatma Phule Krishi Vidhyapeeth, Rahuri.
- Himanen, M. & Hanninen, K. (2011). Composting of bio-waste, aerobic and anaerobic sludges: Effect of feedstock on the process and quality of compost. *Journal of Bioresource Technology*. 102(1), 2842- 2852.
- Nelson, D. W. & Sommers, L. E. (1982). Total carbon, organic carbon and organic matter. In: Methods of soil analysis. (Part 2) Chemical and microbiological properties. A. L. Page, R. H. Miller & D. R. Keeney (Eds). Madison, Wisconsin: *American Society of Agronomy*, 2(1), 539-577.
- Ranalli, G., Bottura, G., Taddei, P., Garavani, M., Marchetti, R. & Sorlini, C. (2001). Composting of solid and sludge residues from agricultural and food industries. Bioindicators of monitoring and compost maturity. *Journal of Environmental Science Health*. 36(4), 415-436.
- Sarkar, P., Meghvanshi, M. & Singh, R. (2011). Microbial Consortium: A new approach in effective degradation of organic kitchen wastes. *International Journal of Environmental Science and Development*. 2(3), 67- 71.
- Sharma, B. L., Singh, S. P. & Sharma, M. L. (2012). Bio-degradation of crop residues by *Trichoderma* species vis-a vis nutrient quality of the prepared compost. *Journal of Sugar Technology*. 14(2), 174-180.

Zhang Y., Qinfen, L. & Xuexi, L. (2013). Study on compost conditions and nitrogen changes of poultry dung with banana Tree. *Journal of Advanced Material Resources*. 613, 153-156.

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