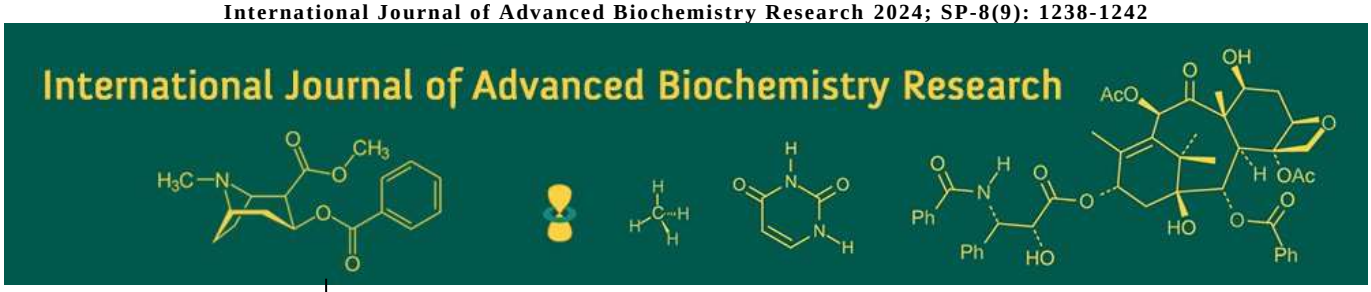

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Exploring cellulolytic enzyme activity of organic matter decomposing fungi *in vitro*

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Abstract
In Konkan region of Maharashtra, traditional methods are used for managing rice residue, finding of an efficient fungal strain to speed up decomposition is essential. In this study, nine fungal species were isolated from compost and mango and cashew leaf litter. *Trichoderma* sp. aff. *T. pseudokoningii* and *Trichoderma* sp. 1 were isolated from mango and cashew leaf litter, while *Aspergillus tubingensis*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3, and *Penicillium* sp. 1 were isolated from compost samples collected from Agronomy and Dairy farms.Among the nine fungal isolates, only four exhibited cellulolytic enzyme activity on Czapek's Dox agar amended with carboxymethyl cellulose. *Aspergillus tubingensis* had the highest cellulolytic index of 0.59, with a clear zone of 50.0 mm. *Trichoderma* sp. aff. *T. pseudokoningii* and *Aspergillus fumigatus* had indices of 0.16 and 0.145, respectively, while *Aspergillus terreus* had the lowest index of 0.125.

Keywords: Decomposition, cellulolytic enzyme activity, carboxymethyl cellulose, cellulolytic index, decomposing fungi

Introduction
Globally, India is one of the culturally diverse country and home of nearly 1.4 billion people. Agriculture is the backbone of Indian economy with more than 15 per cent share in national gross domestic product (GDP). In India, annually 127 Mt of residue is generated taking average harvest index of 0.45 (Dutta *et al*., 2022). Organic wastes take much time to decay and so farmers prefer to use this agricultural waste for fuel purpose only. It is well known that organic matter in the form of crop residue has a profound effect in physical, chemical and biological properties of soil. The decomposition of organic material is important regulating factor of nitrogen, sulfur and phosphorus cycling, given their presence in a number of important organic compounds (Anonymous, 2014) [1].
Cellulose is the most abundant chemical constituents of plant litter. It consists of glucose units, often thousands of units in length, but none of this glucose is available to support microbial metabolism until acted upon by exoenzymes. Cellulose breakdown requires three separate enzyme systems (Paul and Clark 1996) [6]: Endocellulases break down the internal bonds to disrupt the crystalline structure of cellulose. Exocellulases then cleave off disaccharide units from the ends of chains, forming cellobiase. Some soil microbes, including most fungi, produce the entire suite of cellulase enzymes. Other organism, such as some bacteria, produce only some cellulase enzymes and must function as part of microbial consortia to gain energy from cellulose breakdown.

Materials and Methods
The present investigation was laid out during 2023-24 in Completely Randomized Design (CRD) with 9 treatments and each treatment replicated five times at Plant Pathology laboratory, Department of Plant Pathology, College of Agriculture, Dapoli, Dist. Ratnagiri (M.S.). The nine different fungal species were successfully isolated from samples of compost and mango and cashew leaf litter. *Trichoderma* sp. aff. *T. pseudokoningii* and *Trichoderma* sp. 1 were isolated using the standard tissue isolation method from mango and cashew leaf litter.

In compost samples collected from Agronomy and Dairy farms, College of Agriculture, Dapoli, seven fungal species were identified: *Aspergillus tubingensis*, *Aspergillus terreus*, and *Aspergillus fumigatus* were isolated from the Agronomy farm, while *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3, and *Penicillium* sp. 1 were obtained from the Dairy farm using the serial dilution method.

A preliminary qualitative analysis for cellulolytic activity was conducted by using the method of Hankin and Anagnostakis (1977) [3]. Cellulolytic activity was determined based on formation of clear zone and cellulolytic index on CMC plate media.

The detailed procedure as below:

1. The fungi were inoculated aseptically in Petri plates already poured with sterilized CMC amended Czapek's mineral salt agar medium.
2. The plates were labelled with each fungus.
3. The fungi were inoculated on labelled plates.
4. The inoculated plates were incubated at 27± 2 °C in BOD incubator for 4 days.
5. At the end of incubation, to visualize the hydrolysis zone, the agar medium was flooded with an aqueous solution of Congo red (1% W/V) for 15 min.
6. The Congo red solution was then poured off and the plates were further treated by flooding with 1M NaCl solution for 15 min.
7. The cellulolytic index was measured to select the highest cellulase activity producer. The highest cellulolytic index was assumed to contain the highest activity.
8. In order to increase zone contrast, 1 M HCl was added to the plate at room temperature for 10 min, and pour off. The background of the plate was turned into blue.

The cellulolytic index was calculated by using the following formula

$$\text{Cellulolytic index} = \frac{\text{Clear zone diameter} - \text{Colony diameter}}{\text{Colony diameter}}$$

Treatment details

Tr. No.	Fungal isolates
T_1	*Trichoderma* sp. aff. *T. pseudokoningii*
T_2	*Trichoderma* sp. 1
T_3	*Aspergillus tubingensis*
T_4	*Aspergillus terreus*
T_5	*Aspergillus fumigatus*
T_6	*Aspergillus* sp. 1
T_7	*Aspergillus* sp. 2
T_8	*Aspergillus* sp. 3
T_9	*Penicillium* sp. 1

Experimental Results

The cellulolytic enzyme activity of fungi was tested *in vitro* by preliminary qualitative analysis by using the method of Hankin and Anagnostakis (1977) [3]. Nine species of fungi were tested on Czapek's mineral salt agar medium containing carboxy methyl cellulose (CMC). The size of colony was measured and stained with Congo red (1% W/V) to visualize zone. The cellulolytic index was measured to select the highest cellulase activity producer. The results obtained thereof are depicted in the Table 1, Plate I and Fig. 1 and narrated here under.

Table 1: *In vitro* testing of enzyme activity of isolated fungi

Tr. No.	Fungal isolates	Clear zone Diameter (mm)	Colony Diameter (mm)	Cellulolytic index
T_1	*Trichoderma* sp. aff. *T. pseudokoningii*	32.5	28.0	0.160
T_2	*Trichoderma* sp. 1	NM	58.0	NM
T_3	*Aspergillus tubingensis*	50.0	31.4	0.590
T_4	*Aspergillus terreus*	45.0	40.0	0.125
T_5	*Aspergillus fumigatus*	22.0	19.2	0.145
T_6	*Aspergillus* sp. 1	NM	30.0	NM
T_7	*Aspergillus* sp. 2	NM	15.0	NM
T_8	*Aspergillus* sp. 3	NM	33.0	NM
T_9	*Penicillium* sp. 1	NM	20.0	NM
	S.E. (m) ±	1.017	1.120	
	C.D. at 1%	4.202	4.309	

NM: Not measurable (Fungi didn't shows clear zone, so it was not possible to calculate the cellulolytic index)

The data presented in Table 1 revealed that, *Aspergillus tubingensis* demonstrated the highest cellulolytic index of 0.59 with clear zone of 50.0 mm and a colony diameter of 31.4 mm. *Trichoderma* sp. aff. *T. pseudokoningii* showed 0.16 cellulolytic index with clear zone of 32.5 mm and a colony diameter of 28.0 mm, while *Aspergillus fumigatus* shows cellulolytic index of 0.145 with clear zone of 22.0 mm and a colony diameter of 19.2 mm. *Aspergillus terreus* exhibited the lowest cellulolytic index of 0.125 with clear zone of 45.0 mm and a colony diameter of 40.0 mm. *Trichoderma* sp. 1, *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3 and *Penicillium* sp. 1 didn't exhibit clear zone, so the cellulolytic index could not be determined.

In order to evaluate cellulolytic enzyme activity of fungi, Manju and Bishnoi (2016) [5] isolated 40 fungi which they screened out qualitatively for their cellulolytic activity. They found that all the fungal isolates had cellulolytic activity in qualitative screening. Waing *et al.*, (2015) [6] isolated different 30 species of fungi present in leaf litters of forest. Out of 30 species, 22 can degrade cellulose as shown by the formation of clear zone around the colony of the organism. Ray and Rath (2007) [7] reported eleven fungi isolated from soil and compost for their cellulase production. Eight fungi viz., *Aspergillus niger*, *A. flavus*, *Alternaria tenius*, *Curvularia masculans*, *Helminthosporium* sp., *Penicillium* sp., *Trichoderma viride* and *T. harzianum* were found to be positive for cellulase production showing zone of hydrolysis on Czapek's mineral salt agar plates. *T. viride* and *T. harzianum* showed higher activity with 64 and 62 mm clearance zone, respectively. Khokhar *et al.* (2012) [4] isolated 17 fungal species from different sources belonging to three genera i.e. *Trichoderma*, *Aspergillus* and *Penicillium*. Cellulolytic fungi were evaluated after 7 days for the production of cellulolytic enzymes by staining with 1% Congo red. The diameter of clear zone on fungal plates, gave an approximate indication of cellulase activities.

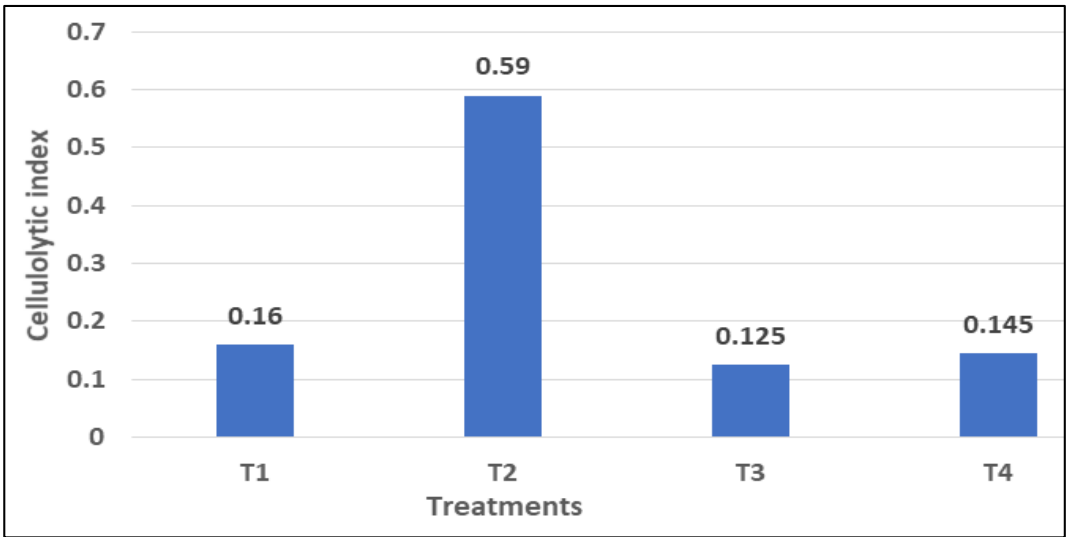
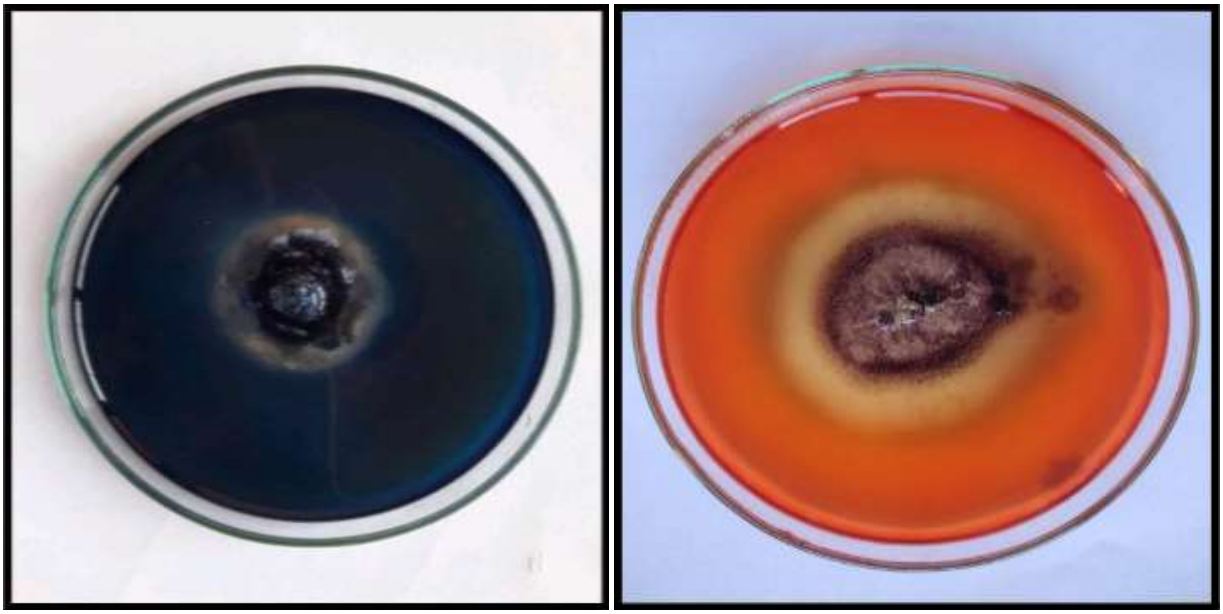


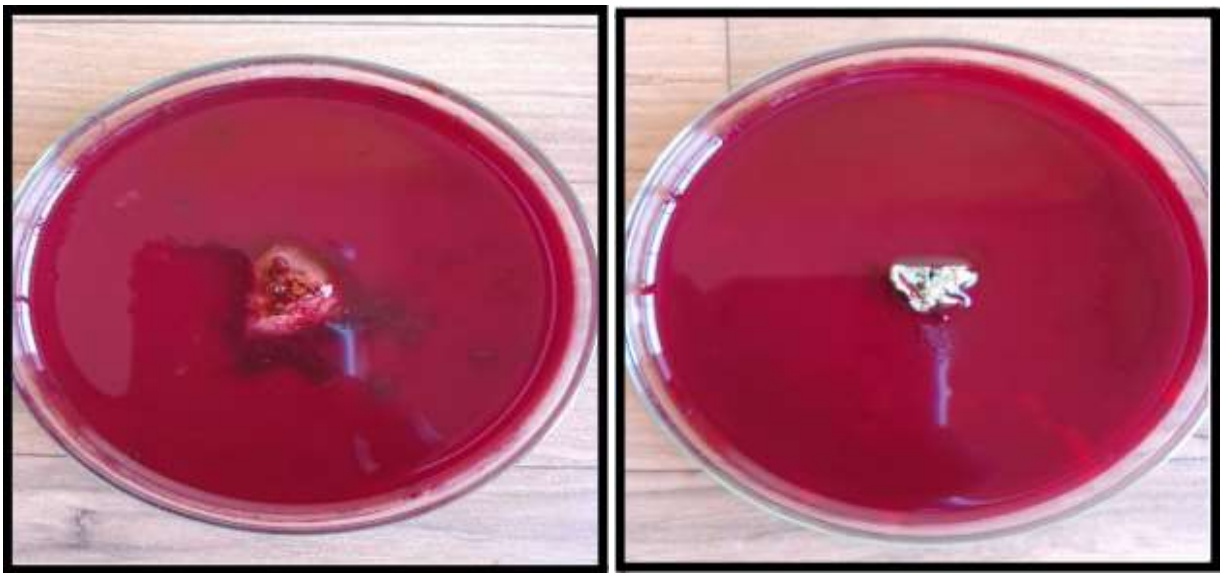
Fig 1: Cellulolytic index of isolated fungi



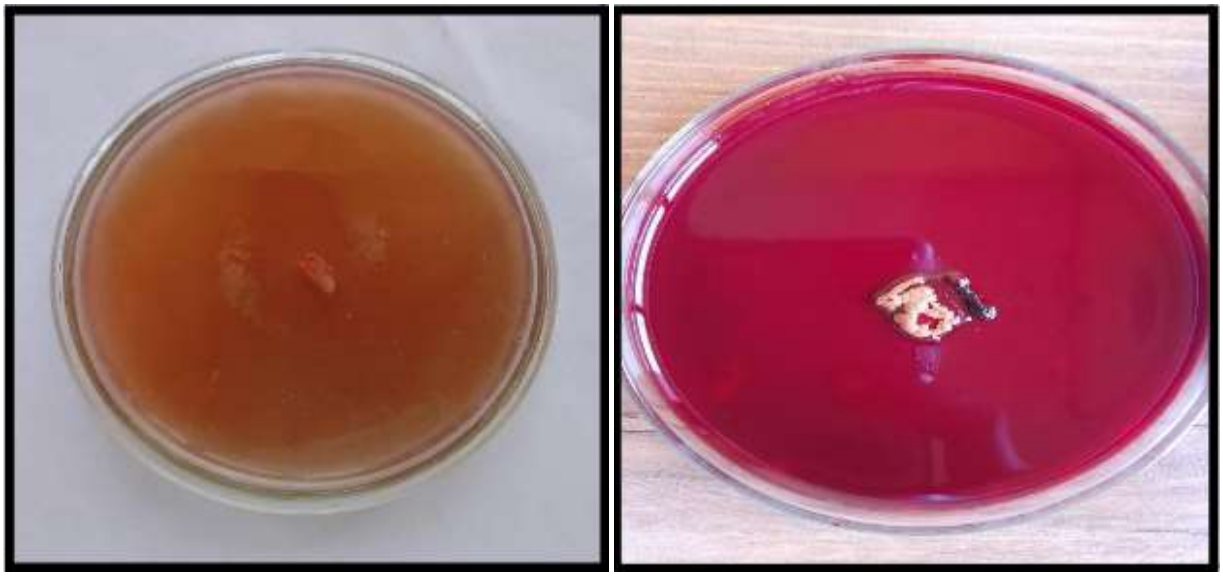
a) *Trichoderma* sp. aff. *T. pseudokoningii* b) *Aspergillus tubingensis*

c) *Aspergillus terreus* d) *Aspergillus fumigatus*

Plate I: Cellulolytic enzyme activity of *T. pseudokoningii*, *Aspergillus tubingensis*, *Aspergillus terreus*, *Aspergillus fumigatus*



e) *Aspergillus* sp. 1 f) *Aspergillus* sp. 2



g) *Aspergillus* sp. 3 h) *Penicillium* sp. 1

i) Trichoderma sp. 1

Plate 2: Cellulolytic enzyme activity of *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3, *Penicillium* sp. 1, *Trichoderma* sp. 1

Conclusion

Among the nine fungal isolates, only four demonstrated cellulolytic enzyme activity on Czapek's Dox agar medium amended with carboxymethyl cellulose. *Aspergillus tubingensis* exhibited the highest cellulolytic index of 0.59, with a clear zone of 50.0 mm and a colony diameter of 31.4 mm. *Trichoderma* sp. aff. *T. pseudokoningii* showed a cellulolytic index of 0.16, with a clear zone of 32.5 mm and a colony diameter of 28.0 mm. *Aspergillus fumigatus* had a cellulolytic index of 0.145, with a clear zone of 22.0 mm and a colony diameter of 19.2 mm. *Aspergillus terreus* exhibited the lowest cellulolytic index of 0.125, with a clear zone of 45.0 mm and a colony diameter of 40.0 mm.

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References

1. Anonymous. [Internet]; c2014. Available from: http://www.nerrs.noaa.gov/doc/siteprofile/acebasin/html/biores/decomp.
2. Dutta A, Patra A, Hazra KK, Nath CP, Kumar N, Rakshit A. A state of the art review in crop residue burning in India: Previous knowledge, present circumstances and future strategies. Environmental Challenges. 2022;1(8):112-128.
3. Hankin L, Anagnostakis SL. Solid media containing carboxymethylcellulose to detect Cx cellulase activity of microorganisms. Microbiology. 1977;98(1):109-115.
4. Khokhar I, Haider MS, Mushtaq S, Mukhtar I. Isolation and screening of highly cellulolytic filamentous fungi. Journal of Applied Sciences and Environmental Management. 2012;16(3):223-226.
5. Manju, Bishnoi NR. Isolation, screening and identification of enzyme producing fungi isolated from degraded wood, fruits and animal dung. International Journal of Advanced Multidisciplinary Research. 2016;34:78-82.
6. Paul EA, Clark FE. Soil Microbiology and Biochemistry. 2nd ed. San Diego: Academic Press; c1996.
7. Ray P, Rath S. Occurrence of cellulase in soil fungi. Journal of Microbial World. 2007;9(1):172-174.
8. Waing KGD, Gutierrez JM, Galvez CT, Undan JR. Molecular identification of leaf litter fungi potential for cellulose degradation. Mycosphere. 2015;6(2):139-144.