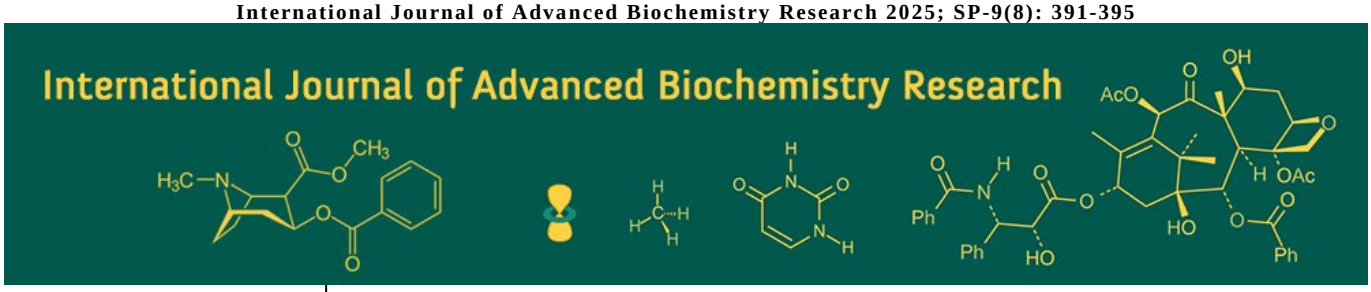

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Changes in enzyme activties during decomposition of crop residues using microbial consortia

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Abstract
An experiment was conducted in 2019 to investigate the dynamics of enzymatic activity during the composting of major crop residues, namely paddy straw, sesame stalks, redgram stalks, and cotton stalks. The study aimed to understand how microbial consortia affect the decomposition process and enzyme production. Two different microbial consortia were employed: Decomposer-1, comprising *Trichoderma reesei*, xylan-degrading bacteria, phosphate-solubilizing bacteria (PSB), and zinc-solubilizing bacteria; and Decomposer-2**,** containing *Aspergillus nidulans*, *Trichoderma viride*, and *Phanerochaete chrysosporium*. A control treatment without microbial inoculation was included for comparison. The results revealed that all crop residues showed significantly increased activities of key enzymes—total dehydrogenase, acid phosphatase, and alkaline phosphatase—when composted with Decomposer-1, peaking at the 30th day of decomposition. After reaching the peak, enzymatic activities gradually declined until the 120th day. Interestingly, paddy straw and sesame stalks exhibited maximum enzymatic activities earlier, at the 30th day, while redgram and cotton stalks reached their highest enzyme activities slightly later, around the 60th day of decomposition. This variation highlights the influence of crop residue type on the rate and efficiency of microbial decomposition. Overall, the application of an effective microbial consortium like Decomposer-1 can accelerate organic matter breakdown and enhance nutrient availability, suggesting a promising strategy for efficient composting and sustainable residue management in agriculture.

Keywords: Composting, consortia, enzyme activities, crop residues, microbes

Introduction
Agricultural crop residues are produced in vast quantities across India and represent a largely underutilized resource of renewable biomass. According to Bhuvaneswari *et al*. (2019) [4], India generates approximately 500 million tons of crop residues annually. Despite this abundance, their full potential remains untapped, particularly in sustainable agricultural and environmental applications. Traditionally, crop residues have been used for animal fodder, fuel, and thatching. However, in recent years, alternative and more sustainable management practices have gained attention, including composting, bioenergy generation, vermicomposting, and mushroom cultivation. Among these, the biological management of crop residues using microbial consortia has emerged as a promising and eco-friendly approach. This method not only aids in efficient biomass degradation but also contributes significantly to soil health improvement, nutrient cycling, and environmental pollution mitigation.

Microorganisms such as bacteria, fungi, and actinomycetes play a vital role in the decomposition of lignocellulosic materials found in crop residues. These microbes secrete specific enzymes that break down complex organic polymers like cellulose, hemicellulose, and lignin into simpler, assimilable compounds. This microbial degradation process can occur under either aerobic or anaerobic conditions, depending on the composition of the microbial community and the environmental parameters involved. The adoption of such bioconversion techniques offers multiple benefits, including enhanced soil fertility, carbon sequestration, improved soil structure, and increased microbial diversity, all of which are key factors in sustaining long-term agricultural productivity (Gurumurthy *et al*., 2018) [10].

In this context, soil and compost enzyme activities are considered reliable indicators of microbial function and organic matter transformation.

Enzymes such as dehydrogenase, phosphatase, and cellulase serve as sensitive markers of microbial activity and changes in soil biological properties resulting from organic residue management practices. As Dick (1992) [8] emphasized, enzyme activity reflects the biochemical functioning of microbial communities and their interaction with organic substrates. Furthermore, factors influencing microbial communities—such as substrate type, moisture, temperature, and external inputs—can directly or indirectly affect enzymatic activity (Tiwari *et al*., 1988; McClaugherty and Linkins, 1990) [13]. Therefore, monitoring enzyme activities during composting not only provides insights into the decomposition process but also helps evaluate the effectiveness of microbial inoculants in accelerating residue breakdown and enhancing compost maturity.

The biological oxidation of organic compounds is generally a dehydrogenation process and there are many dehydrogenases, which are highly specific. The dehydrogenase enzyme system apparently fulfills a significant role in the oxidation of soil organic matter as they transfer hydrogen from substrates to acceptors. Many different specific dehydrogenase systems are involved in the dehydrogenase activity of soil; these systems are an integral part of the microorganisms. Therefore, the results of the assay of dehydrogenase activity would show the average activity of the active population (Skujins, 1976) [16].

Phosphatases encompass a diverse group of enzymes that are found both intracellularly and in the soil environment, where they play a crucial role in phosphorus cycling. These enzymes catalyze the hydrolysis of esters and anhydrides of phosphoric acid, thereby releasing inorganic phosphate from organic compounds (Spier and Ross, 1978) [17]. Phosphatase activity in soil is closely linked to the availability and turnover of organic phosphorus. Both plant roots and soil microorganisms contribute to this process. Plant roots are capable of secreting phosphatases into the rhizosphere, enabling them to mineralize organic phosphorus compounds for uptake. Similarly, a wide range of soil microbes—including bacteria and fungi—also produce phosphatases, enhancing the overall phosphorus availability in the soil system. This microbial contribution is especially important in soils where phosphorus is predominantly present in organic forms, making enzymatic hydrolysis essential for maintaining soil fertility and supporting plant growth.

2. Materials and Methods

2.1 Stubble collection and resizing

Fresh stubbles of cotton, sesamum, redgram and paddy are collected from farm and resized them to 10-15 cm length. These stubbles are stored carefully till the start of the experiment. Initial sample collected to characterize the crop residue and preserved.

2.2 Development of culture

In case of decomposer -1(*Trichoderma reesi*+ Xyline degrading bacterial + PSB + Zinc solubilizing bacteria) mother culture of 20 gms is added to 200 lt of water mixed with 2kgs of jaggery and mixed thoroughly. Stirred the contents daily once with a sterilized wooden stick for a period of 14 days early in the morning.

In case of decomposer-2 (*Aspergillus nidulans*+ *Trichoderma viridae*+ *Phaenerochete chrysogenum*) microbial consortium 100 ml of mother culture is added to 200 lt water mixed with jaggery and mixed thoroughly and stirred the contents daily twice morning and evening with a sterilized wooden stick.

2.3 Bed preparation and addition of microbial consortium

Each bed with a dimension of 2 x 1 m marked. In each bed initially 5 kg of residue saturated with moisture placed and 1 lt of microbial consortium added. Again remaining 5kg of residue saturated with moisture placed above the initial residue and 1lt of microbial consortium spread over it evenly.

2.4 Maintaining moisture content and turning over

Moisture content is maintained at 60% by addition of water at regular intervals. The composting piles were turned fortnightly to maintain aeration.

2.5 Sample collection

The representative samples of compost from each treatment were collected at 30, 60, 90 and 120 days after incubation were analyzed for the enzyme activities such as dehydrogenase and phosphatase (acid and alkaline). Samples were collected from different depths and places of pile and pooled together and were preserved at 4^0C for analysis of microbial activity parameters.

2.6 Enzymatic assays

Dehydrogenase activity in the samples was determined by following the procedure as described by Casida *et al*. (1964) [5]. Phosphatase activity of samples was determined by following the procedure of Eivazi and Tabatabai (1977) [9].

3. Results and discussion

3.1 Dehydrogenase activity (µg TPF $g^{-1}hr^{-1}$)

Dehydrogenase activity, a key indicator of overall microbial oxidative activity in compost, exhibited a dynamic pattern during the decomposition of different crop residues. In the case of paddy straw (S1) and sesamum stalks (S2), dehydrogenase activity increased steadily and peaked on the 30th day of composting, after which it gradually declined until the 120th day (Figure 1). This decline suggests that a significant portion of the easily degradable organic matter had been mineralized by that point, resulting in the formation of more stable humified compounds. Similar trends were reported by Tiquia *et al*. (1996) during the composting of pig-manure and sawdust, where dehydrogenase activity decreased as compost matured.

In contrast, redgram stalks (S3) and cotton stalks (S4) exhibited a delayed peak in dehydrogenase activity, with the highest levels observed around the 60th day of decomposition. This extended activity phase may be attributed to the presence of bulking agents or higher lignocellulosic content, which likely stimulated sustained microbial metabolism and delayed the stabilization process (Ros *et al*., 2006) [15]. Interestingly, redgram and cotton stalks also recorded higher peak activity values than paddy and sesamum, indicating a more prolonged microbial response to their residue composition.

Across all treatments, the application of Decomposer-1 (a consortium of *Trichoderma reesei*, xylan-degrading bacteria, PSB, and zinc-solubilizing bacteria) consistently resulted in the highest dehydrogenase activity. Specifically, maximum activity levels of 62.50 µg TPF g^{-1} hr^{-1} in paddy straw and 52.80 µg TPF g^{-1} hr^{-1} in sesamum stalks were

observed on the 30th day, while redgram and cotton stalks reached 58.23 µg TPF g^{-1} hr^{-1} and 50.65 µg TPF g^{-1} hr^{-1}, respectively, on the 60th day. The elevated enzyme activity in these treatments suggests that the microbial consortium significantly enhanced decomposition by accelerating microbial biomass buildup and metabolic function.

Over time, as the readily available carbon substrates were exhausted, microbial growth and enzyme production declined—a trend supported by previous studies (Castaldi *et al*., 2007) [6]. Since enzyme activity is directly linked to microbial biomass, its decline during the latter stages of composting is a natural outcome of biomass degradation (Ayuso *et al*., 1996) [2]. Conversely, the lowest dehydrogenase activity was recorded in control treatments where no microbial consortia were applied, reaffirming the critical role of microbial inoculants in enhancing compost maturity and efficiency.

3.2 Alkaline phosphatase activity (µg p-nitrophenol g^{-1} h^{-1})

Alkaline phosphatase exhibited high activity during 30th day of composting for paddy straw (S_1) and sesamum stalks (S_2) then, the activity was declined up to 120th day of composting (Fig 2), with, respectively 29.09 - 21.23 µg p-nitrophenol $g^{-1}hr^{-1}$ and 28.24 - 24.67 µg p-nitrophenol $g^{-1}hr^{-1}$ between 30 to 120th day of composting. Whereas, redgram stalks(S_3) and cotton stalks (S_4) exhibited highest alkaline phosphatase activity at 60th day of decomposition and later declined up to 120th day of decomposition, with, respectively 36.23 -27.97 µg p-nitrophenol $g^{-1}hr^{-1}$ and 34.83 - 31.07 µg p-nitrophenol $g^{-1}hr^{-1}$. The high organic matter content and large quantity of nutrients in original compost stimulate growth of total aerobic bacteria and subsequent phosphatase and peptidase synthesis (Cunha-Queda *et al.*, 2007) [7]. Addition of decomposer-1 resulted in higher alkaline phosphatase activity of 36.24 µg p-nitrophenol $g^{-1}hr^{-1}$, followed by 32.99 µg p-nitrophenol $g^{-1}hr^{-1}$ registered with decomposer-2 and the lowest activity of 26.55 µg p-nitrophenol $g^{-1}hr^{-1}$ was noticed in control treatment at 60th day of decomposition. An increased enzyme activity with application of microbial consortia is also an indicator of microbial activity related to the cycling of chemical elements, and significant increases after addition, compared to the control may be due to increased microbial biomass, which may have produced alkaline phosphatase enzymes (Tejada *et al.*, 2006; Bastida *et al.*, 2008) [19, 3]. All the crop residues registered highest total alkaline phosphatase when they were composted with the decomposer 1 and the lowest was registered when the residues were decomposed without any microbial consortia.

3.3 Acid phosphatase activity (µg p-nitrophenol g^{-1} hr^{-1}):

Similar to, alkaline phosphatase, acid phosphatase also gradually increased from the initial stage of composting and exhibited high activity during 30th day of composting for paddy straw (S_1) and sesamum stalks (S_2) then, the activity was declined up to 120th day of composting (Fig 3), with, respectively 30.60- 25.37 µg p-nitrophenol $g^{-1}hr^{-1}$ and 27.90 - 22.83 µg p-nitrophenol $g^{-1}hr^{-1}$ between 30 to 120th day of composting. Whereas, redgram stalks (S_3) and cotton stalks (S_4) exhibited highest acid phosphatase activity at 60th day of decomposition and later declined up to 120th day of decomposition, with, respectively 40.50 - 38.07 µg p-nitrophenol $g^{-1}hr^{-1}$ and 37.30 - 29.57 µg p-nitrophenol $g^{-1}hr^{-1}$. This is due to greater quantities of available substrates in these residues. This is consistent with many studies about increases in acid phosphatase activity resulting from organic matter amendments (Jordan *et al.*, 1995 and Kremer and Li, 2003) [11, 12].

Addition of decomposer-1 resulted in higher acid phosphatase activity of 35.83 µg p-nitrophenol g^{-1} hr^{-1}, followed by 34.70 µg p-nitrophenol $g^{-1}hr^{-1}$ registered with decomposer-2 at 60th day of decomposition. Acid phosphatase activity was increased when the crop residues were treated with microbial consortia compared to control due to more less biological activity. Albrecht *et al.*, 2010 [1] in their study of composting process reported that acid phosphatase activities were clearly related to biological activity and respiration patterns in the compost. All the crop residues registered highest acid phosphatase activity when they were composted with the decomposer-1 and the lowest was registered when the residues were decomposed without any microbial consortia, suggesting that acid phosphatase activity depends on the availability of substrate and microbial population in the compost (Albrecht *et al.*, 2010) [1].

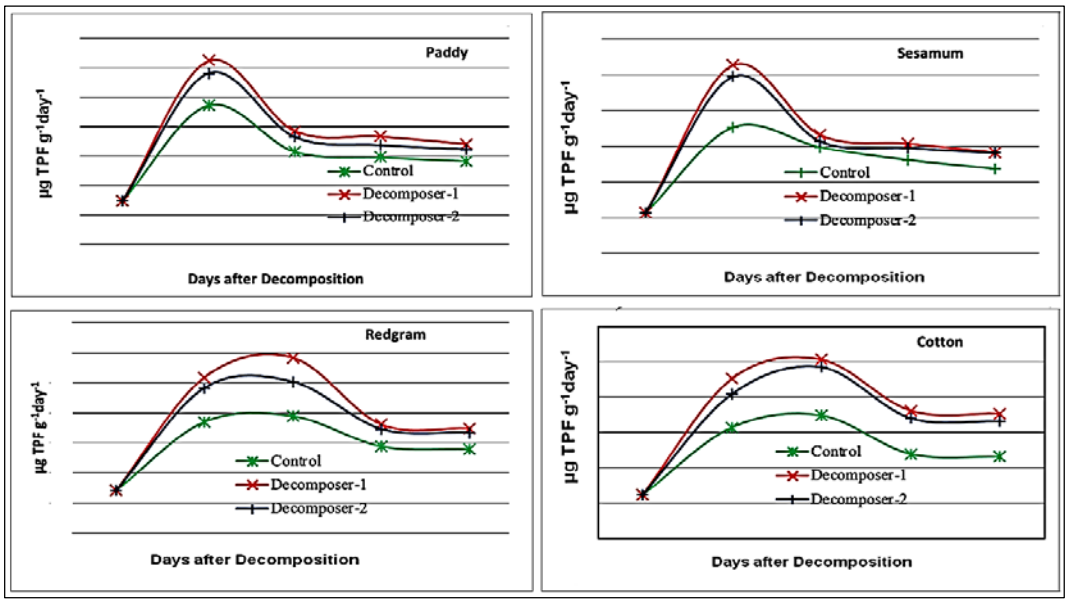

Fig 1: Dehydrogenase activity (µg TPF $g^{-1}day^{-1}$) of composting material as influenced by crop residues and decomposers

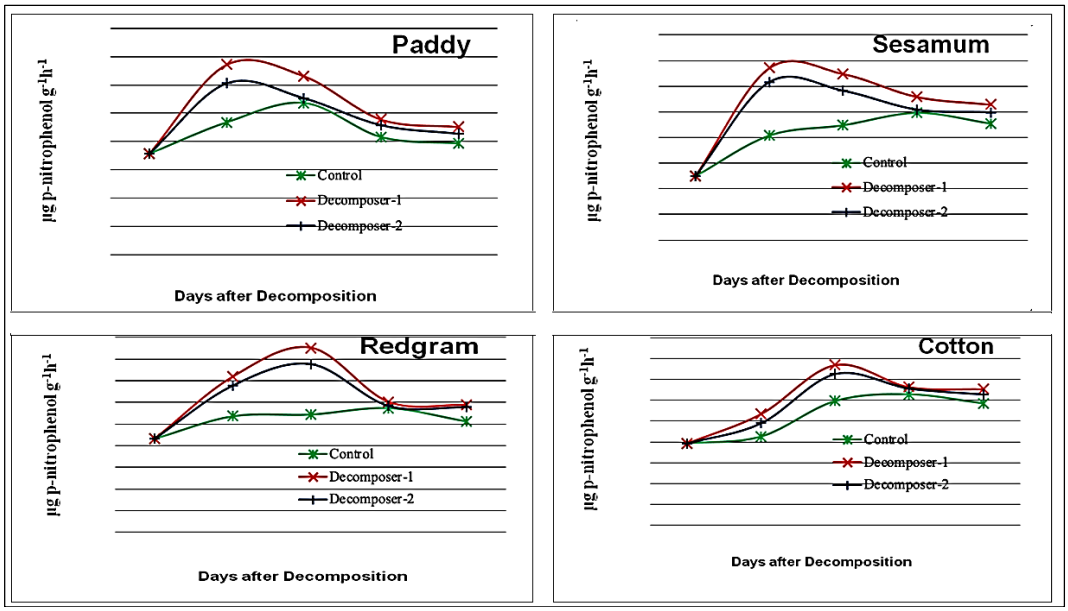

Fig 2: Alkaline Phosphatase activity (µg p-nitrophenol $g^{-1}h^{-1}$) of composting material as influenced by crop residues and decomposers

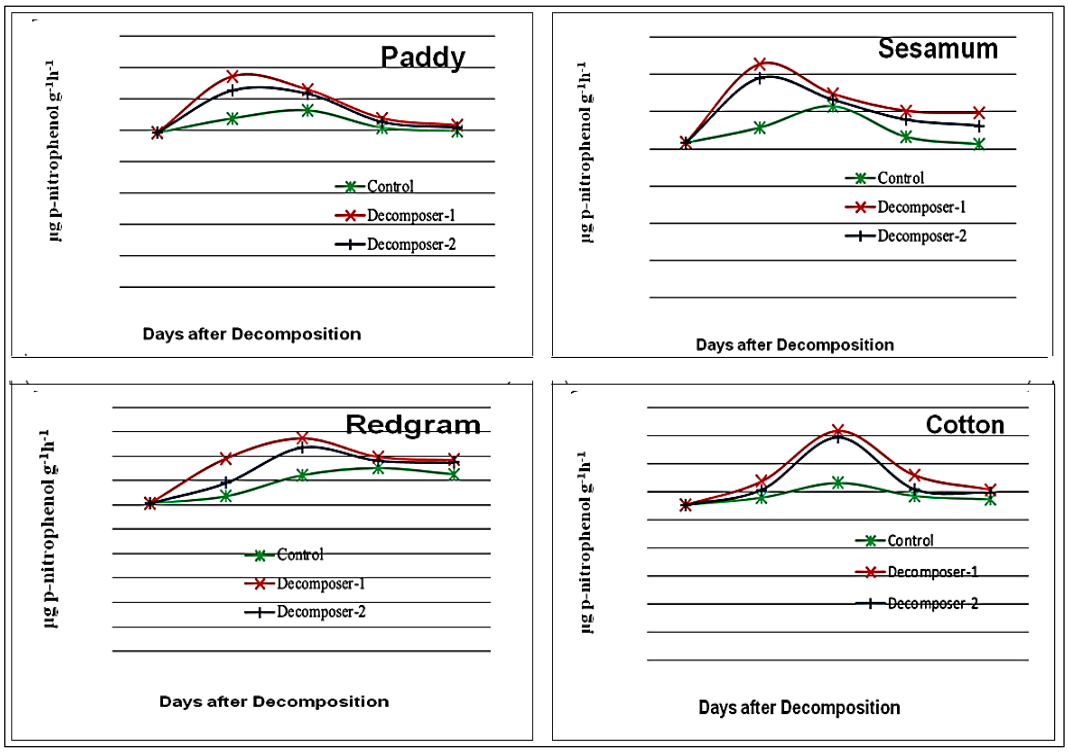

Fig 3: Acid Phosphatase activity (µg p-nitrophenol $g^{-1}h^{-1}$) of composting material as influenced by crop residues and decomposers

Conclusion

From the investigation, it can be concluded that microbial consortia played a vital role in decomposition of residues at faster rates, improving the enzymatic activity of compost. All crop residues treated with decomposer-1 exhibited higher enzymatic activity than decomposer-2 followed by control.

Future Scope

Decomposition of crop residues using microbial consortium in the field itself after the harvesting of crops, so as to study the effectiveness at field level and also hastening the process of decomposition through external application of nitrogen to narrow the C:N ratio, thus faster mineralisation of nutrient and making them available to plant.

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