Screening and application of cellulolytic fungal strains for maize straw composting: Effect on Physico-Chemical Properties and Enzymes Activity

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Abstract: The sustainable agriculture is mandatory to meet up the food product market demand in any developing country. It not only includes higher production but also recycling and reuse of waste or byproducts. The presented investigation includes such recycling of maize straw waste for compost development which can be further utilized for higher crop production. The maize straw alone, with cow dung and with or without cellulolytic fungal isolates inoculations was utilized for development of compost. All the raw materials of maize waste, cow dung and soil were analyzed, and screening of soil was done for isolation of cellulolytic fungal strains which were determined as *Aspergillus niger* and *Aspergillus proliferance.* The various combinations with or without inoculation of cellulolytic fungal strains analyzed for composting periodically in which all physical parameters viz. pH, temperature, moisture content and electrical conductivity and enzymes i.e. CMCase and xylanase activity were analyzed which showed inoculation of fungal consortium positively affect the compost physiology.

Key words: maize straw recycling, sustainable agriculture, cellulolytic fungal strains.

Introduction:

The recycling of agricultural wastes is an inherent part of the sustainable agriculture and a wide area of investigation. There were many crops, vegetables and fruits waste were utilized for composting to obtain good quality fertilizer as well as to get rid of agricultural waste. The composting is an eco-friendly technology, it is amongst the most effective methods for farming agro-waste management, capable of achieving high performance biotransformation. Composting is an organic reaction that occurs by means of microbial community, encompassing the mesophilic, thermophilic, and mature stages. Meanwhile, it is an essential option for the long-term agro by-products viz. biogas.

Maize straw is a low-cost and renewable lignocellulosic material. The global yearly supply of maize straw has already been estimated to be over 1 billion tonnes. Traditionally, large volumes of maize straws are burning in the field, resulting in nutritional losses and severe environmental damage. As a result, it is vital to seek an environmentally beneficial approach of dealing with maize straws that minimises harmful environmental consequences. The breakdown of maize straw contributes significantly to global carbon cycle. Microorganisms serve critical functions

in the composting of maize straw. Understanding microbial characteristics and activities in response to seaweed fertiliser amendment aids in our understanding of microbial roles and the identification of appropriate management techniques. During the composting process, the functions of microbial community regulated organics decomposition, humic-like substance formation, and nutrient transformations are comparatively well managed to understand, but there is little available information about microbiological functional characteristics, particularly carbon utilisation capacity.

Identifying keystone species that play a more vital role in organic waste breakdown throughout the composting process may be helpful for improving our understanding of the biodegradation. Thus, presented article revealed the screening, application and attributes of cellulase producing fungal strains during the maize straw waste composting.

Materials and methods:

The agricultural waste maize straw and fresh cow dung were collected locally from Jepura, Halol, Panchmahals, Gujarat, India. Maize straw (1-3 cm) was moistened with water and analysed by physio-chemical analysis before constructing compost pile.

2.1 Physio-chemical analysis of feed stock samples:

Various physico-chemical analysis of compost were carried out viz., pH, Temperature, Moisture and Electrical Conductivity (EC) were analysed. pH was measured using glass electrode of Systronic MK VI.

2.2Experimental set-up: Composting was conducted at a location with complete shadowing to limit moisture loss. Compost bin were prepared. Cow faeces and maize straw were piled high in the bin. To achieve better homogeneity, the cow dung and maize straw were physically combined. Twelve separate combinations (bins 1 to 12), designated here as T1 to T12 were made with the following ingredients: T1 and T2 contained respective maize straw and cow dung alone, respectively; T3- Maize straw + *Aspergillus niger*; T4- Maize straw + *Aspergillus proliferans*; T5- Maize straw + *Aspergillus niger* + *Aspergillus proliferans*; T6- Cow dung + *Aspergillus niger*; T7- Cow dung + *Aspergillus proliferans*; T8- Cow dung + *Aspergillus niger* + *Aspergillus proliferans* T9- Maize straw + Cow dung; T10- Maize straw + Cow dung + *Aspergillus niger*; T11- Maize straw + Cow dung + *Aspergillus proliferans*; T12- Maize straw + Cow dung + *Aspergillus niger* + *Aspergillus proliferans*.

Fungal strains: From seven lignocellulolytic fungal strains, based on their enzyme's catalytic reaction with pure substrate, two strains, *Aspergillus niger* and *Aspergillus proliferans* were selected for application in composting. These two strains were maintained on PDA slants and stored at 4°C.

Inoculum preparation:

For inoculation, seed inoculum was prepared using salt medium as described by Ekperigin (2007). The 100 mL prepared seed inoculum was diluted with 400 mL distilled water. The inoculums of *A. niger* and A. *proliferans* were sprayed on the composted materials. The inoculum was spread as layer.

2.3 Turning: The content in each bin was turned manually at 15 days intervals and moisture content was maintained 70% by spraying water.

2.4 Sampling of composts and analysis of produced compost:

The 100g samples were taken at 30, 60 and 90 days as per the method described by Ishil and Takii (2003). Various physico-chemical viz., pH, Temperature, Moisture, EC, CMCase and Xylanase of compost samples were analysed as per standard methods. Further enzymatic activity and compost maturity were analysed. For the enzymatic characterization the sample was kept at 4°C. 5g of produced composts were extracted from the compost pit with 25 ml citrate buffer (pH 4.8; 50mM) by shaking the mixture at 180 rpm for 30 minutes, then filtered using Whatman No 2-filter paper. The filtrate was utilised in enzyme assays.

Xylanase: The activity of Endo-1,4-xylanase was evaluated by combining 1.0 mL of 1 percent (w/v) birch wood xylan with 0.1 mL of 0.05 M citrate buffer (pH 4.8) and incubating the mixture at 50°C for 30 minutes. By adding 1 mL of DNS reagent, the process was halted. At 540 nm, the resulting decreasing sugar was detected (Miller, 1959). The enzymatic activity required to release 1 mol of xylose equivalents per unit volume per minute of reaction was defined as one international unit (IU).

CMCase: 0.5 mL filtrate having crude enzyme, 1.0 mL 1 percent CMC, and 1.0 mL 0.05 M (pH 4.8) sodium citrate buffer (w/v) were added to the mixture and incubated for 30 minutes at 50°C. The reaction was stopped by adding 1 mL DNS reagent to the mix. At 540 nm, the resulting decreasing sugar was detected (Miller, 1959). The enzymatic activity required to release 1 mol of glucose equivalents per unit volume per minute of reaction was defined as one international unit (IU).

Results and discussion:

3.1 Analysis of raw materials for composting:

The raw materials were analyzed to check the physico-chemical properties which further utilized for composting. The parameters of Maize straw were parallel to Zhou et al., (2019); Glab et al., (2018) and Zhong et al., (2011) with 5 to 10 % of variation. The soil parameters were in accordance of Gondek et al., (2018); Glab et al., (2020); Zhang et al., (2011). The cow dung parameters were parallel to Ezekoye and Ezekoye (2009).

The experiments were governed in from of duplicates and results were represented as Mean±SD

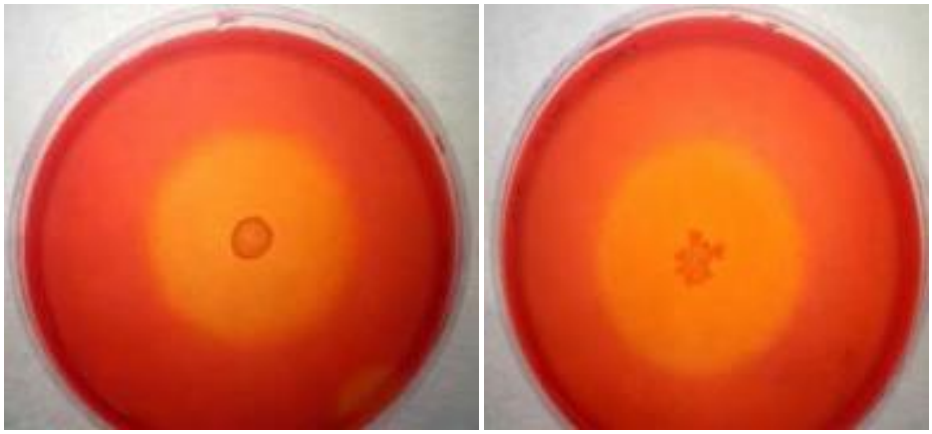
3.2 Screening of predominant fungal strains from the Maize straw dumping site:

The fungal strains were utilized for composting due to their capability to penetrate the solid substrates and ability to produce higher content of degrading enzymes than the bacteria. Primary screening was done on PDA agar following repeated subculturing to get pure colonies. The 7 filamentous fungal isolates (5mm disc) were transferred to CMC agar plates to check their cellulose degrading capability which reflected in terms of production of extracellular cellulase enzyme (Table 1). The zone of cellulose solubilization was checked by 0.1% Congo red and higher degrading enzyme producer strains were selected for further use (Figure 1).

Table1. Analysis of cellulase producers by zone of solubilization on CMC agar plates

Sr. No.	Isolate No.	Zone of solubilization in mm
1	IS1	6.0±0.50
2	IS2	15.4±0.45
3	IS3	25.6±1.20
4	IS4	32.0±1.46
5	IS5	10.7±0.70
6	IS6	9.57±1.00
7	IS7	8.83±0.67

The experiments were governed in form of triplicates and results were represented as Mean±SD

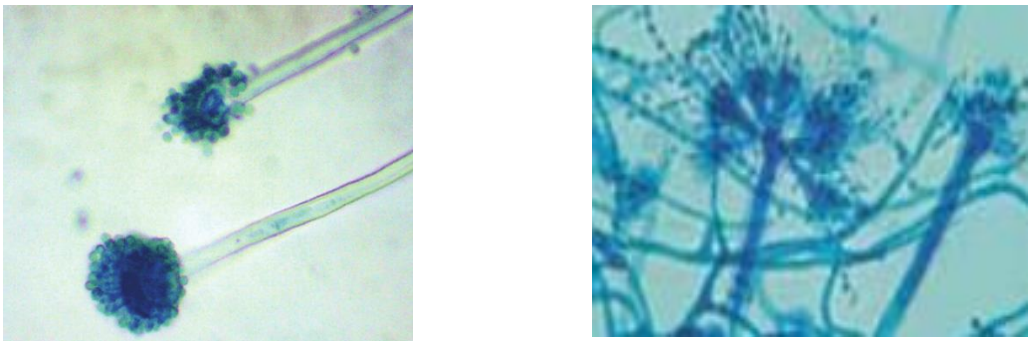


IS3 **IS4**

Figure: 1 The cellulase production by higher producer fungal isolates

Identification and characterization of selected fungal isolates:

The two fungal isolates were screened which were characterized by microscopic (Figure 2). The identified fungal strains were *Aspergillus niger* and *Aspergillus proliferance*.



IS3 **IS4**

Figure 2 The microscopic images of IS3 and IS4 fungal strains

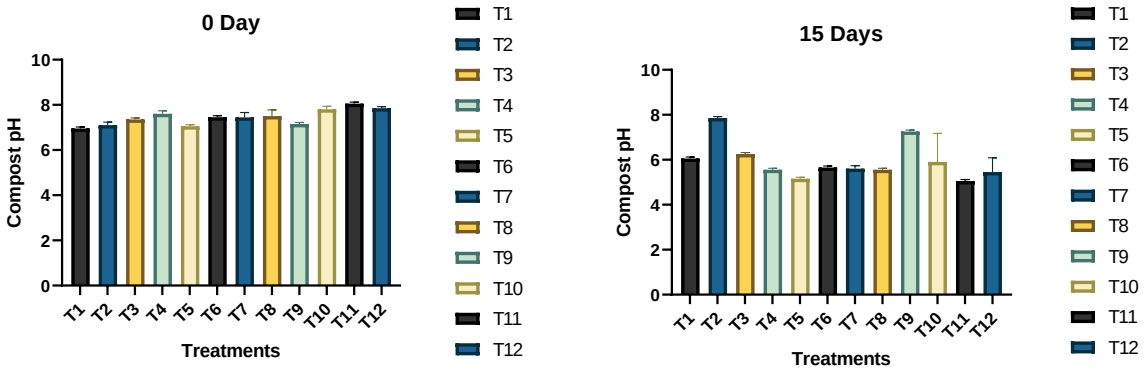
3.3 Physicochemical characterization of compost formulated by different treatments:

3.3.1Analysis of physical parameters of compost:

pH and temperature of compost: The pH and temperature of compost was analyzed periodically by collecting samples from three different locations of pile. The pH was decreased at 15 days and become acidic due to release of organic acids during active decomposition. At later stage of composting, it increased by 60 days due to ammonia production and most highest variation was reported in case of fungal consortium inoculated in mixture of maize straw and cow dung comprising pile. pH was stabilized at maturity stage due to vitalization of nitrification

and ammonia and become near to neutral (Figure 3). The pH range of final compost obtained was in accordance with outcome of Gondek et al., (2018).

The temperature was also checked periodically on 0, 15, 60 and 90 day time. The temperature raised in thermophilic phase and followed by lowering of temperature called as mesophilic phase which was become stable at the maturity. The present range and pattern of variation in temperature was matched with Wan et al., (2020). Consequently, the treatments in which maize straw was mixed with cow dung revealed high temperature rise initially. The maximum lowering of the temperature was reported in case of treatments having only maize straw material for composting. The inoculation of consortium of *Aspergillus* fungal strains affected the temperature modulation via increasing the decomposition rate in thermophilic phase than the other treatments.



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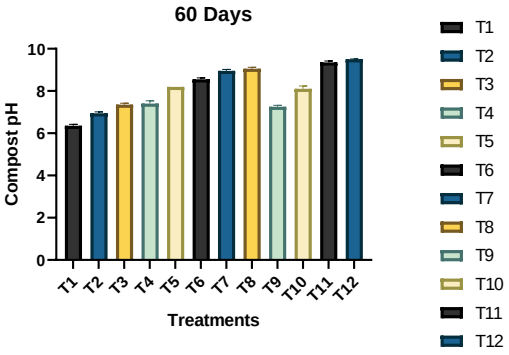

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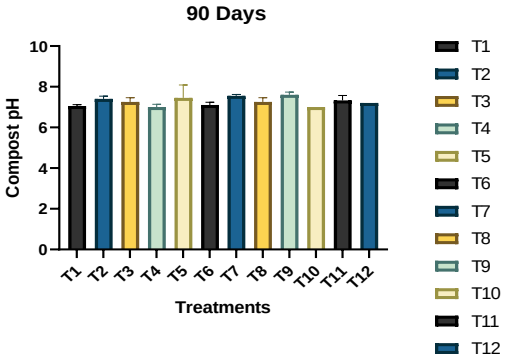

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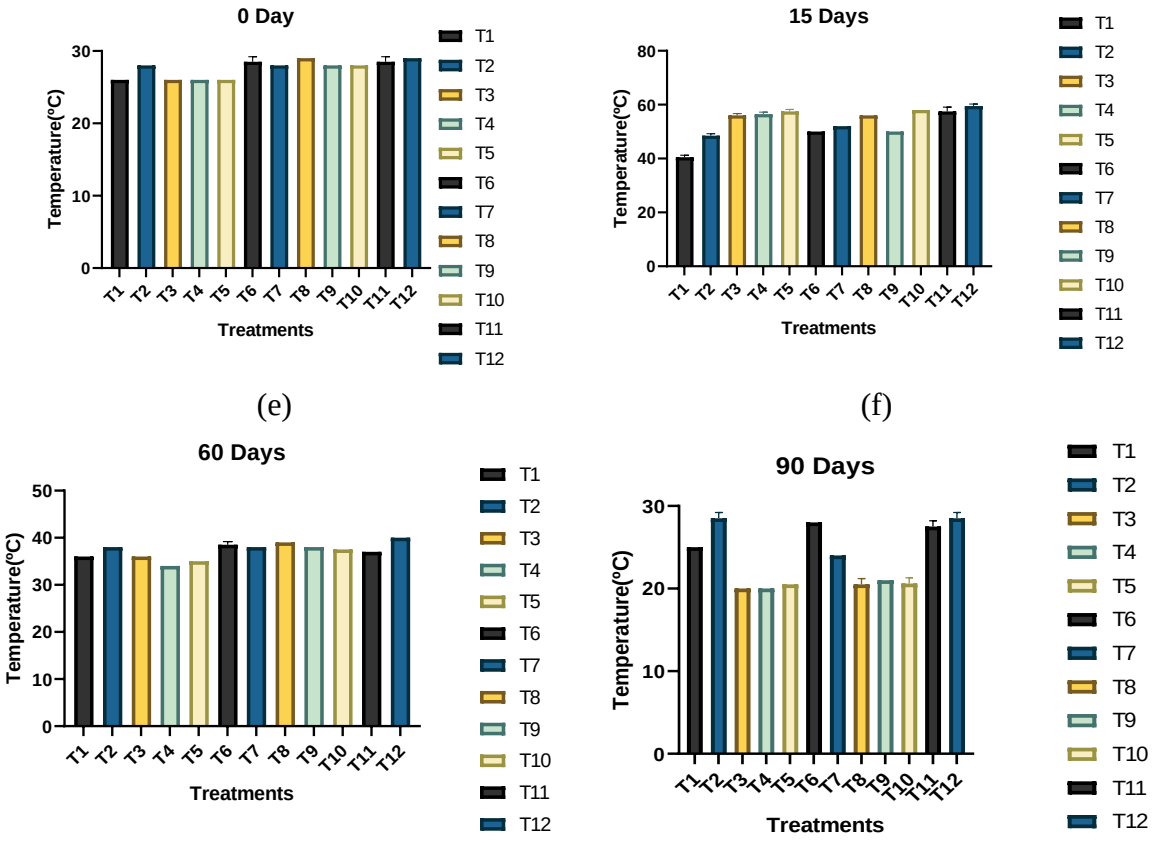


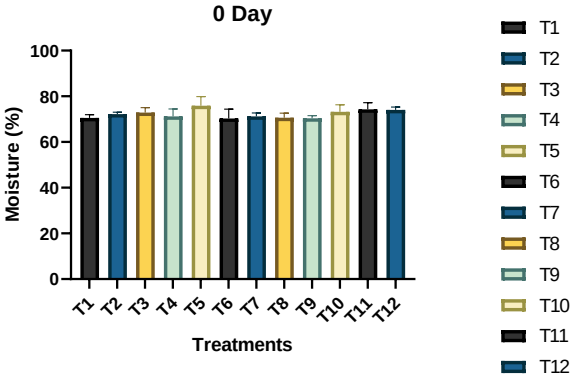
Figure 3. Analysis of effect of various treatments on compost pH at (a) 0 day, (b) 15 days, (c) 60 days, (d) 90 days, and compost temperature at (e) 0 day, (f) 15 days, (g) 60 days, (h) 90 days

Compost moisture and EC content:

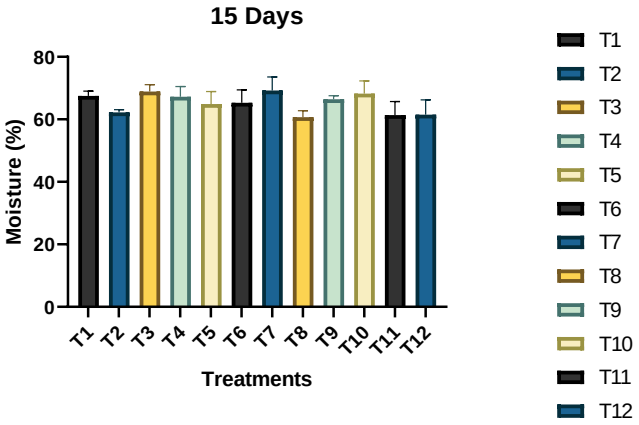
The physical parameters viz. moisture content and EC value denoted the quality of compost. The initial moister content in compost pile should be in range of minimum 40 to 50% which gets reduced in thermophilic phase due to raise in temperature which was maintained by watering the pile for optimum decomposition and microbial activity. It was again increased at later stage due to decomposition of material and release of water in the compost. The initial moisture content minimum of 70% provide good condition for degradation of hardly degradable organic compounds.

The electrical conductivity is the measure of salinity of the compost. During composting, the increase in it showed the progressive mineralization of organic matter. Release of such minerals was the highest in Maize straw compost than cow dung compost whereas incorporation of cow dung in Maize based compost reduce it at intermediate level (Figure 4). The results are in accordance of Gondek et al., (2018); Glab et al., (2018). The incorporation of fungal isolates

also affected the moisture content and electrical conductivity in which the inoculation of both the strains as consortium in compost pile increase the moisture content than the other treatments which was due to higher mineralization of compost materials.



(a)



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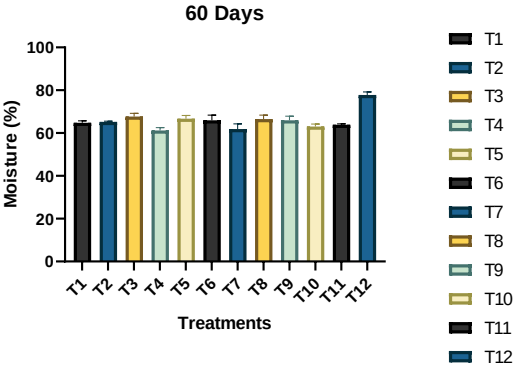

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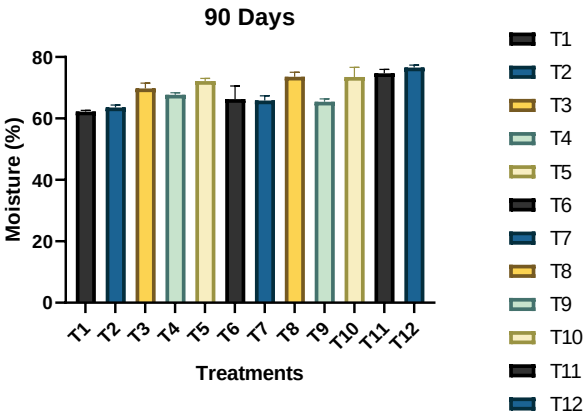

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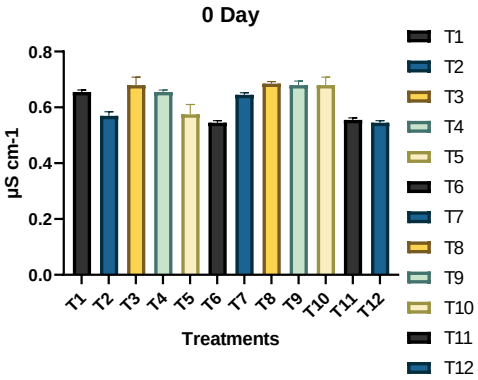

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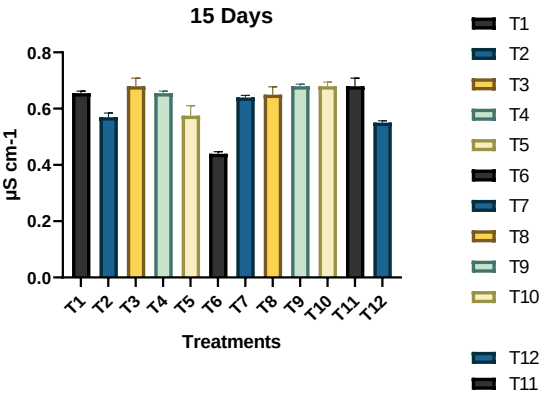
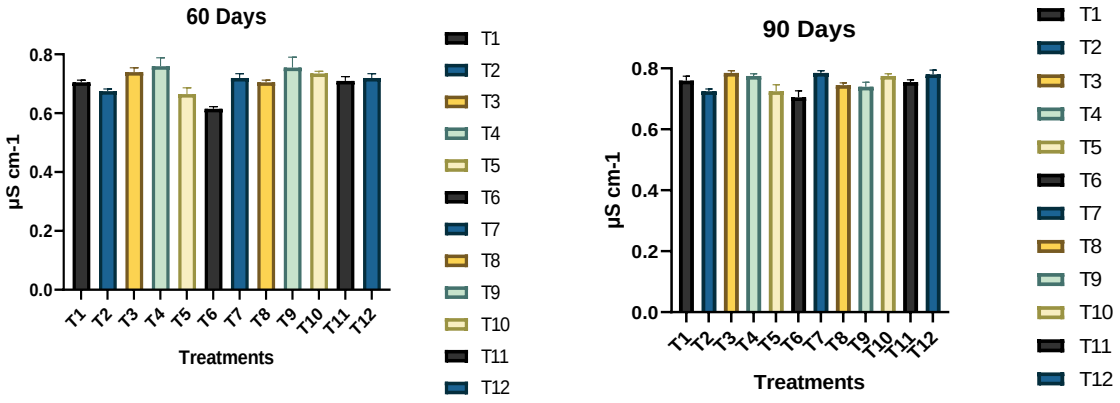


(f)



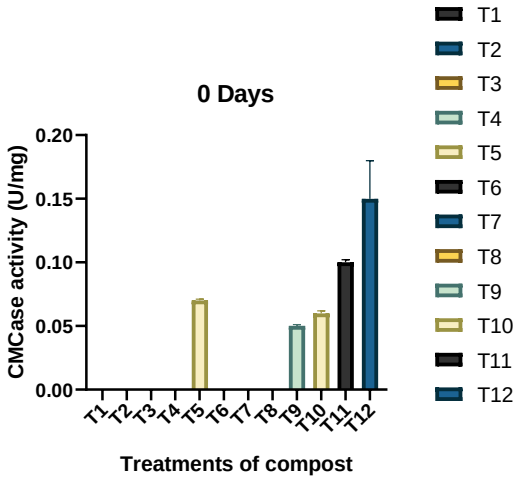
(g) (h)

Figure 4. Analysis of effect of various treatments on compost moisture content at (a) 0 day, (b) 15 days, (c) 60 days, (d) 90 days, and compost EC value e) 0 days, (f) 15 days, (g) 60 days and (h) 90 days

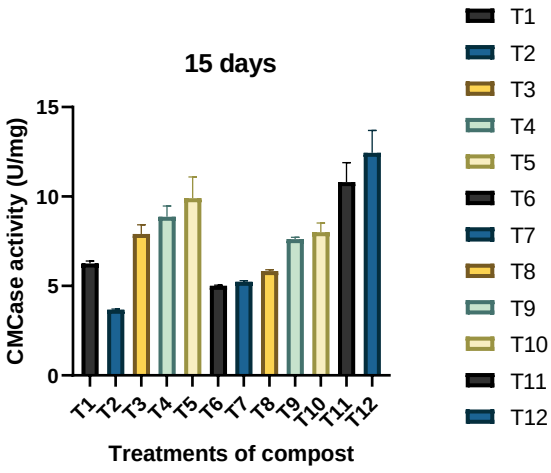
3.3.2Analysisof chemical parameters of compost upon various treatments:

CMCase and xylanase activity: CMCase is the one type of cellulase which degrades the cellulose whose decomposition is considered as the most important rate limiting step of compost formation. The active metabolism of cellulose was reported for 15 days of composting which leads to active degradation of substrate and growth of microorganisms simultaneously (Figure 5). The microbes produce the inhibitors of CMCase (Heilmann-Clausen and Boddy, 2005) which later reduced its activity which was also reflected in the presented study. The enzyme activity was higher in case of maize straw comprising treatments than the sole cow dung due to high availability of cellulose and hemicellulose which was similar to Raut et al., (2008). Goyal et al., (2005) investigated that the higher nitrogen availability promotes the growth of microflora which stimulate synthesis of CMCase and similar raise upon 75 and 90 days. The main substrate for xylanase secretion is hemicellulose which was degraded at a very high rate at thermophilic phase and thus, at 15 days the maximum enzyme activity was obtained.

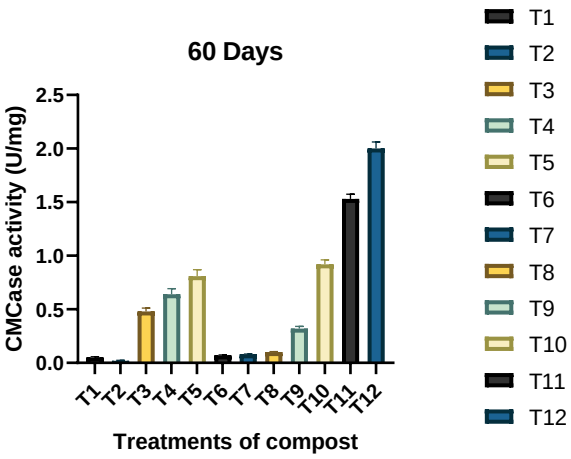
The pattern of xylanase enzyme activity was similar to CMCase (Zeng et al., 2010). The variation with time in xylanase activity was due to microbial growth and high nitrogen availability (Paola et al., 2008; Castaldi et al., 2008). The decline in enzyme activity also revealed the maturity of compost.


0 Days
CMCase activity (U/mg)
0.20
0.15
0.10
0.05
0.00
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12
Treatments of compost
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12

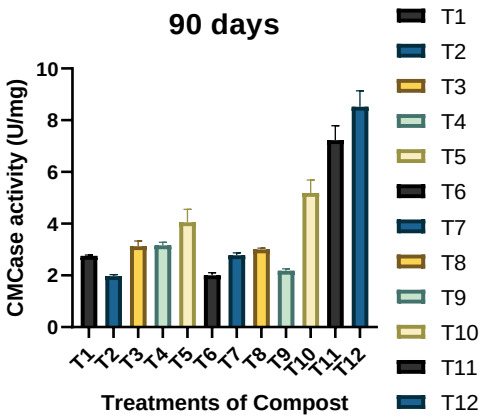
(a)


15 days
CMCase activity (U/mg)
15
10
5
0
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12
Treatments of compost
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12

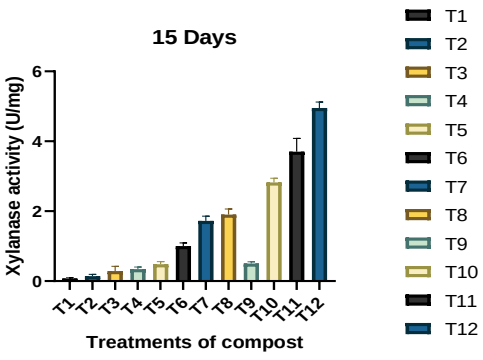
(b)


60 Days
CMCase activity (U/mg)
2.5
2.0
1.5
1.0
0.5
0.0
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12
Treatments of compost
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12

(c)


90 days
CMCase activity (U/mg)
10
8
6
4
2
0
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12
Treatments of Compost
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12

(d)


15 Days
Xylanase activity (U/mg)
6
4
2
0
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12
Treatments of compost
T1
T2
T3
T4
T5
T6
T7
T8
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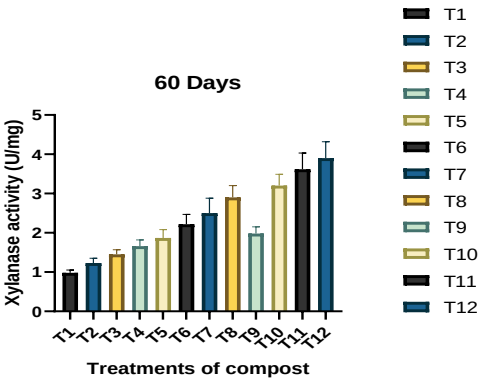
T10
T11
T12

(e)


60 Days
Xylanase activity (U/mg)
5
4
3
2
1
0
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12
Treatments of compost
T1
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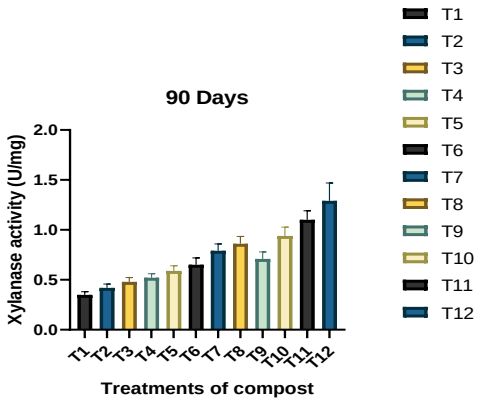
T10
T11
T12

(f)



(g)

Figure 5. Analysis of CMCase enzyme activity at different time points (a) 0 day, (b) 15 days, (c) 60 days, (d) 90 days, and xylanase activity at (e) 15 days, (f) 60 days (g) 90 days from compost piles having differently formulated composts

Conclusion: The presented investigation shown that the cellulolytic fungi can be suitably utilized for composting of maize straw composting. The physicochemical as well as maturity parameters were affected by the inoculation of separate and consortium of both fungal strains. The maize straw and cow dung in mixed form when inoculated with the consortium of both the strains of fungal isolates the most desirable quality comprising compost was formulated. The composting from such agriculture wastes was not phytotoxic and thus suitable for sustainable agriculture.

Acknowledgement-

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