Original Research Paper

Volume - 16 | Issue - 03 | March - 2026 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Agricultural Science

ROLE OF CELLULASE PRODUCING *TRICHODERMA* SPECIES IN SUSTAINABLE BIOFERTILIZER PRODUCTION FOR SALINE SOIL MANAGEMENT

Shailaja Dhutekar* Research Scholor Department of Microbiology, Rajarshi Shahu Mahavidyalaya Autonomous, Latur-413512, Maharashtra India*Corresponding Author

Deepak Vedpathak Associate Professor Department of Microbiology, Rajarshi Shahu Mahavidyalaya Autonomous

Rahul More Assistant Professor Department of Microbiology, Dayanand Science College, Latur-413512, Maharashtra India

ABSTRACT The accumulation of agricultural waste (agro waste) and soil salinity are major constraints to sustainable agriculture, necessitating eco-friendly solutions for waste degradation. This study investigates the biodegradation potential of salt-tolerant Trichoderma species and their enzymatic activity in decomposing organic matter under saline conditions. Five salt tolerant Trichoderma isolates previously isolated and identified from mangrove soil Vikhroli, Mumbai. *(T. viride, T. harzianum, T. atroviride, T. longibrachiatum, and T. koningii)* were screened for cellulase production. Among them, *T. viridae* (T1), and *T. harzianum* (T2), showed maximum cellulase activity, with the highest enzyme production recorded at pH 5 temperature 32^{0}C, time duration 5 days and substrate concentration 20g/L at 3% salt concentration for both Trichoderma sp. confirming their potential in breaking down cellulosic materials. Biodegradation trials demonstrated a significant reduction in dry weight, with dark brown coloration, indicating effective organic matter decomposition. Effect of biodegradation product on plant growth promotion of *Triticum aestivum* and *Cicer arietinum* showed significant increase than control. These results highlight the enzymatic efficiency of 3% salt-tolerant *Trichoderma* species in biodegrading agro waste and improving soil fertility. The findings support the application of *Trichoderma* mediated biodegradation as a sustainable alternative for organic waste recycling, soil health restoration, and biofertilizer development in salt-affected agricultural regions.

KEYWORDS : Biodegradation, Salt tolerant *Trichoderma spp.*, Cellulase, Biofertilizer

INTRODUCTION

Soil salinization is a major environmental challenge that threatens global agricultural productivity, affecting approximately 20% of irrigated lands worldwide (Khan *et al.* 2010). Organic amendments such as bio-fertilizer, vermicomposting, vermiwash, seed priming and application of growth regulators can improve the salinity tolerance of agricultural plants, leading to increased yields. Microbial biodegradation has emerged as a promising strategy for converting agricultural residues into bioavailable nutrients, reducing waste, accumulation, and improving soil health (Zhao and Zhang, 2015).*Trichoderma species* are well known for their ability to degrade lignocellulosic materials through the secretion of hydrolytic enzymes, such as cellulases and laccases, which facilitate organic matter decomposition and nutrient availability (Hasan et al. 2014). Additionally, these fungi play a crucial role in soil fertility enhancement by decomposing organic matter.

The integration of *Trichoderma*-mediated biodegradation into agricultural systems aligns with the principles of sustainable land management and circular economy. By transforming agricultural waste into bio fertilizers, these fungi not only reduce environmental pollution but also enhance soil organic matter content, leading to improved crop productivity in stress-prone environments (Ilangumaran, Smith et al. 2017). *Trichoderma*-based biofertilizers were found to enhance soil microbial activity, leading to increased nutrient uptake and improved plant health (Iqbal *et al*. 2020).

Despite these promising applications, the full potential of *Trichoderma*-mediated biodegradation products in saline soil remains underexplored. This study aims to produce biofertilizer using biodegradation of agrowaste which can be effective in promoting plant growth in saline soils. Hence, providing a sustainable alternative to chemical fertilizers and conventional soil amendments.

MATERIALS AND METHODOLOGY

Collection of soil samples from salt stressed ecosystem

A soil sample was collected from the Mangrove area in Vikhroli, Mumbai latitude 19.105270^{0}N and longitude 72.934372^0 E during the months of February to March 2022. The samples were procured from the depth of 7-10 cm of rhizospheric regions from soil surface near the plants.

Isolation of Trichoderma spp. from saline soil

The isolation of Fungi was carried out directly after the preparation of the samples by using a modified method described by (Ajijolakewu *et al.,* 2013). Different media used such as Potato Dextrose Agar (PDA) medium supplemented with 0.05 g/L chloramphenicol to inhibit bacterial growth and Rose Bengal agar (RBA) and *Trichoderma* selective media/L.with 3% and 4% salt concentrations. The plates were incubated at 28^{0}C for 5–7 days to allow fungal colony development. For morphological identification, hyphal morphology and colony growth patterns of salt-tolerant *Trichoderma* isolate were observed at 72, 96, 120, 144, and 168 h post inoculation on PDA, synthetic low nutrient agar (SNA), and corn meal dextrose agar (CMD) (Zhang C. *et al* 2022). After inoculation on SNA and CMD media for 72 and 168 h, characteristics of the conidia, conidiophores, chlamydospores were examined and photographed under optical microscope. Then growth pattern was compared with a published description. The strains were maintained on potato dextrose agar with 3% and 4% salt concentration (PDA) and stored at 4^{0}C until further use.

Agricultural Waste Substrate

Lignocellulosic agricultural waste was collected from different place in Latur, Maharashtra, India. Four samples were collected from the fields. Agricultural residues, including wheat straw, chickpea straw, sugar cane bagasse of sugar cane waste and leaves and stem samples of trees were collected in sterile polyethylene bags, dried, and ground into small particles (~2 mm) for further biodegradation studies.

Screening of cellulase Producing *Trichoderma* Isolates

Qualitative Screening by using carboxymethylcellulose (CMC) – agar

The cellulase activity of the isolates was determined by the CMC hydrolysis test. The fungal cultures were inoculated onto CMC agar plates and incubated at 28^{0}C for 5 days. After incubation, the plates were flooded with 0.1% Congo red solution for 15 minutes, followed by washing with 1M NaCl. The formation of clear zones around the colonies indicated cellulase production. The diameter of the clear zones (in mm) was recorded. (Ajijolakewu *et al.,* 2013)

Confirmatory test for cellulase activity by using the agro- wastes

Individually, 5g from each waste was replaced in the CMC-agar medium as the sole carbon source. Then, all the media sterilized at 121°C for 15 minutes. An inoculum from each isolate culture was separately inoculated on the modified CMC-agar medium; then

incubated at 28^{0}C for 5-7 days in inverted position. All the samples prepared in Triplicates. After incubation time, all the plates stained with 1% (w/v) Congo-red solution, then left for 15 minutes at room temperature and finally washed with 1 M NaCl for 15 minutes. The plates examined by the naked eye for the presence of clear zones that formed around the fungal colonies; the isolates that formed the highest clear zone were selected. (Sirisena, Manamendra., *et al* 1995)

Molecular identifications of selected *Trichoderma* isolates

Trichoderma isolate selected for better performance in cellulase enzyme producing activities was further subjected for molecular identification. Flask containing 3% salt PDB medium was inoculated with 5 mm mycelial plug from the margins of freshly grown isolates of T1 and T2. Post incubation, (at 28^{0}C for 5 days) mycelium was harvested and .Genomic DNA was extracted using the CTAB (Cetyl trimethyl ammonium Bromide) method with minor modifications by (White *et al*., 1990). The purity and concentration of DNA were determined using a Nano drop spectrophotometer (A260/A280 ratio) and gel electrophoresis on a 1% agarose gel. The Internal Transcribed Spacer (ITS) region of rDNA was amplified using universal primers: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') by PCR reactions. The thermocycler was programmed with initial denaturation at 95 ◦C for 5 min followed by 30 cycles of 4 min, annealing at 55 ◦C for 45sec, elongation period at 72 ◦C for 1 min and a final extension step of 10 min at 72 ◦C. The PCR product were resolved on a 1.5% agarose gel stained with ethidium bromide and visualized under a UV Tran illuminator. Successful amplicons (~550 bp) were purified using a PCR purification kit (Qiagen, USA) and sequenced by Sanger sequencing. The obtained sequences were compared with reference sequences in the NCBI GenBank database using BLAST analysis. Phylogenetic trees were constructed using the Neighbour-Joining method in MEGA software (version 11.0) with bootstrap support of 1000 replicates to determine evolutionary relationships among isolates.

Optimization of Cellulase Production

Optimization of cellulase activity culturing condition was carried out by using a modified method described by (Agarwal *et al.* (2014) and Sharma and Sharma (2016). Agro waste (which representing the enzyme substrate) was used as the sole C- source instead of the CMC and 3% NaCl. *T.viride* and *T.herzianum* separately and a consortium of these two selected *Trichoderma* isolates was used. The test were carried out at different pH (5, 7, 8 and 9), Time durations (3, 4,5and 7days), Temperature (27^{0}C, 32^{0}C, 37^{0}C, and 40^{0}C) and substrate concentrations (5g, 10g, 20g and 30g) under 3% salt concentration. Quantitative evaluation of the cellulase activity when the optimized growth conditions for the isolated *Trichoderma viridae* KY566166, *Trichoderma harzianum* AF278790 collected in one test by using the agricultural wastes as C-source

The biodegradation efficiency of the *Trichoderma* isolates and their consortium (T1 and T2) was assessed using optimized enzymatic activity conditions that resulted from the previous tests and control medium with uninoculated *Trichoderma* consortium using agricultural waste as C source under 3% saline conditions. The cellulase activity was assayed as mentioned before.

RESULTS AND DISCUSSION

Accumulation of the agricultural waste is the major problem associated with environmental pollution. Other treatment options are costly than utilization and bioconversion of those waste in to high value industrially useful product. Using the potato dextrose agar and Rose Bangal agarmedia and cultivation conditions described in the above section, a total 17 different fungal isolates were recovered from Mumbai mangrove area soil samples. Out of five fungal spp three were 3% salt tolerating and two were 4% salt tolerating spp showed morphological similarity with *Trichoderma* spp (Mohanavely et al, 2021)). Morphological characteristics of five *Trichoderma* spp were showed in Table 1. The ability of those selected five *Trichoderma* isolates to produce cellulase enzyme was determined qualitatively.

Table 1: Morphological Characteristics of *Trichoderma* Isolates

Isolate	Colony color	Colony Reverse color	Conidiophore	Phialide character	Conidia Shape
T1	Dark Green	Amber Pale yellowish	Frequently branching and verticillate	Frequently paired,lageniform &verticillate	Ellipsoidal
T2	Dark green	Dull yellowish	Rarely branched and verticillate	Cylindrical or slightly inflated And divergent	Ellipsoidal
ST1	Dark green cottony texture	No colour	Branched, pyramidal or irregular shape	Langiform or ampulliform	Sub globose
ST2	Green to White	No colour	Branched,pyramidal or irregular shape	Ampulliform	Green globus
SC8	Yellowish green	No colour	Branched,pyramidal or irregular shape	Ampulliform	Green globus

Cellulose is the majour componant of most of the plants cellwall.cellulose is hydrophilic material, but due to it's large size makes it less soluble in water.Crystalline nature of cellulose makes it more resistant to biological degradation (Wagnar,et al 2018)).Here in our study, The mechanical grinding is the mechanical pretreatment followed by thermal treatment (1.5 bar at 1200C) during sterilization of media componants were applied.This increases the surface area of agrowaste by the size reduction (Zheng et alm 2014 & Nishant et al,2017)Which will increase the microbial accessisability to cellulose present in the agrowaste.

The qualitative and quantitative analysis of each Trichoderma isolates were deteced through studying it's ability to hydrolyze different cellulose containing media as asole source of carbon.The simplest source of cellulose is carboxymethylcellulose(CMC) was firstly used as a sole carbon source followed by culturing two trichoderma isolates on modified carboxymethylcellulose (CMC), in which carboxymethylcellulose was replaced with the lignocellulosic agrowaste and 3% salt. The cellulose degrading isolates were detected through formation of inhibition zone upon staining the culture plates with the congo red.

According to the obtained results the finially selected 3% salt tolerating were occurred upon those gave hydrolytic activity with the agrowaste carbon sources.They were selected for further investigations.The cellulase activity was determined by quantitatively by determiningthe amount of those produced reducing sugar/moleto finially choose the encoded isolate with the highest cellulase activity. Screening for cellulase activity by Five *Trichoderma sp.* on CMC agar were shown as, maximum cellulase activity was reported by Trichoderma viride (20mm) followed by Trichoderma harzianum (17mm) in comparison to other spp. (Figure no.1)

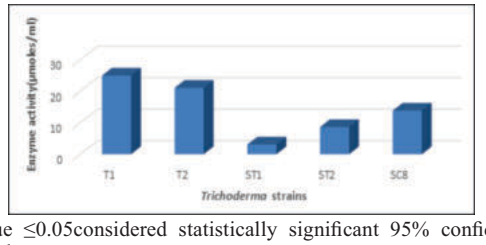
Table 2: Screening of *Trichoderma* isolates for cellulase production on 3% & 4% PDA plates

Trichoderma Isolates code	Cellulase Zone(mm)
T1	20.33
T2	17.66
ST1	8.33
ST2	5.66
SC8	14.66

They were estimated for their cellulase activity through following equation. (Koehler et al. 1976)

$$\text{Enzyme activity} = (T\text{-}C) \times \text{Factor} \times \frac{\text{Volume of Sample}}{\text{Volume of Standard}}$$

Where: T: the O.D. of the test sample, C: the O.D. of the control



P-value ≤0.05considered statistically significant 95% confidence interval

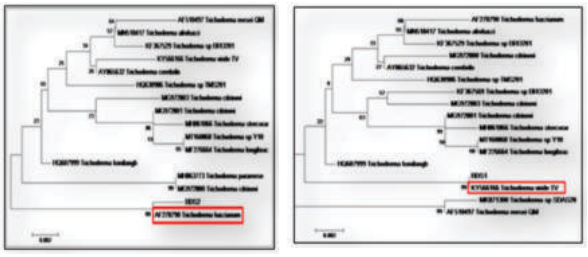
Figure 1: Quantitative analysis of cellulase activity by using agricultural waste as a sole C-source in 3% &4% salt conditions.

It was found the encoded two fungal strains T1 & T2 gave the highest

hydrolytic activity with the agricultural waste. The enzymatic activity were 25 µmoles/ml and 21.5 µmoles/ml. respectively when used separately for biodegradation. For better biodegradation activity a consortium was used.

The encoded two fungal isolates were examined for their identification. At first the fungal isolates were examined for microscopic observations. There after the two fungal isolates were examined for their molecular characterization using 18srRNA. The phylogenetic relationship of these species were blasted with other closely related fungal species present in the GeneBank. The fungi were sequenced as illustrated in figure 2.The tree showed 99% similarity with related genera. The sequence were submitted to NCBI under accession no PQ824499 and PQ821177. (Figure 3 a, b)

Figure 2: Molecular identification of Maximum cellulase producer Trichoderma sp. a) The phylogenetic tree for the Trichoderma harzianum strain AF278790 b) The phylogenetic tree for the Trichoderma viridae strain Ky566166

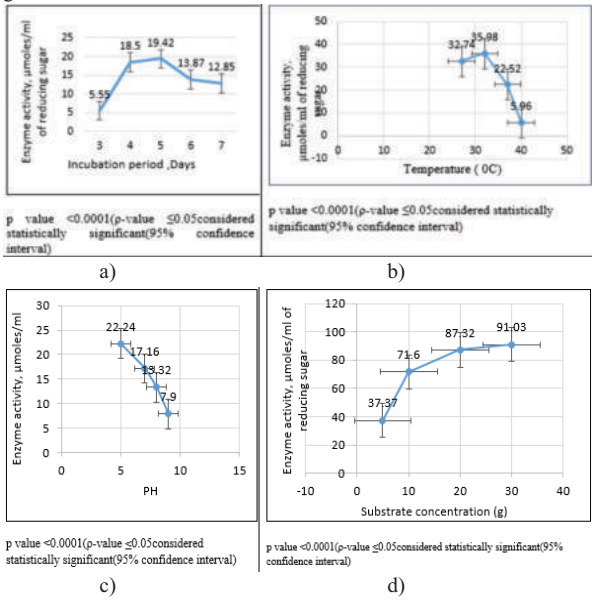


a) b)

Optimization parameters of cellulase production in terms of time duration, pH, and temperature and agro substrate concentration were as: cellulase production increased over time, reaching its peak on Day 5, with the highest enzyme activity (19.42 µmoles/mL). The optimal temperature 320C, yielding the highest enzyme activity (35.98 µmoles/ml), pH 5 being the optimal, yielding the highest enzyme activity (22.24 µmoles/ml) (Ghose T.K, 1987).The study further revealed that substrate concentration significantly influenced biodegradation efficiency, 10 20g as the optimal substrate range for effective biodegradation by Trichoderma species, the maximum sugar release (492µg/ml) occurred at 30g, indicating extensive carbohydrate breakdown. As showed in Figure 4

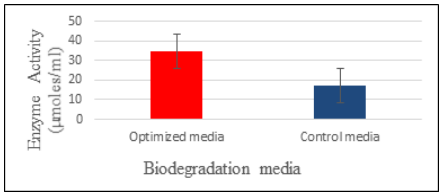
These results are supported by (Kumar and Sharma et al., 2020), who reported that optimal substrate concentrations improve microbial degradation efficiency, whereas excessive substrate levels may hinder fungal activity due to nutrient imbalances or limited oxygen availability.

Figure 3: Optimization of parameter for cellulase production a) Time in days b) Temperature in 0C c) pH d) Substrate concentration in grams.



In a test that designed by the author, the resulted optimum pH, substrate concentration, temperature and the incubation time were collected in one test to determine their effect on the cellulase activity. It was found that, the cellulase activity was increased 34.83 µmoles/ml to reducing sugar /ml.(Figure 4)

Figure 4: The cellulase activity after optimized growth conditions and control collected in one test and by using the agricultural wastes as C-source



P value <0.0001(ρ-value ≤0.05considered statistically significant (95% confidence interval)

Biodegradation of agro waste was achieved by degradative activity of consortium of *Trichoderma viride* and *Trichoderma harzianum* after one month of incubation: the humus obtained from the test sample was dark brown in colour & without odour in comparison to light brown colour of humus from control giving slight odour. (Narendra Kumar et al, 2024) The values of temperature, weight loss and moisture contents of humus obtained from test sample were 450C, 142.9 % and 42 % respectively while the values of temperature, weight loss and moisture contents of humus obtained from control were 370C, 9.3 % and 38 % respectively. Thus consortium of *Trichoderma viride* and *Trichoderma harzianum* was reported very effective in producing humus of good quality from total degradation of agro waste.(Table 3)

Table 3: Characteristics of Biodegradative product after one month of incubation at 30 ^{0}C

Characteristics	Color	Odor	Temperature (0C)	Weight loss %	Moisture %
Test	Dark Brown	No	45	142.9	42
Control	Light Brown	Slight	37	9.3	38

Overall, our results confirm that salt-tolerant *Trichoderma* species play a role in waste biodegradation, making them valuable biofertilizers for saline-affected agricultural systems. These findings reinforce the potential of *Trichoderma*-based biodegradation strategies by promoting soil fertility and enhancing crop productivity in stress-prone environments.

CONCLUSION

Out of five examined *Trichoderma* isolates, the cellulose degrading *Trichoderma viridae* strain KY566166 and *T. herzianum* strain AF278790 exhibited the highest cellulase activity among them. The strain sequencing was submitted to Gene Bank database at NCBI under accession numbers PQ824499 and PQ821177, respectively. The humus obtained from the test sample showed good quality compared to control. These two strains were more effective in biofertilizer production when used in consortium than used separately. These findings reinforce the sustainable role of *Trichoderma*-mediated biodegradation in improving soil fertility and plant health.

REFERENCES

1. Agarwal, T.; Saxena, M. K. and Chandrawat M. P. S (2014). Production and optimization of cellulase enzyme by Pseudomonas aeruginosa MTCC 4643 using sawdust as a substrate. International Journal of Scientific and Research Publications, volume 4(1). ISSN 2250-3153.
2. Ajijolakewu, K. A.; Sani, A.; Oyeyiola, G. P.; Ahmed, R. N.; Arekemase, M.O.; Odebisi-Omakanye, M. B. and Laba, S.A. (2013). Cellulase production potentials of the microbial profile of some sugarcane bagasse dumping sites in ilorin, Nigeria. Notulae Scientia Biologicae, 5(4): pp. 445-449.
3. Ghose T.K. (1987).Measurement of cellulase activities, Pure and applied chemistry, 59(2), 257-268.
4. Hasan, M. M. (2014). Biological control of plant pathogens with Trichoderma species. Journal of Plant Pathology & Microbiology, 5(11), 1–4.
5. Narendra Kumar H. K.; Chandra Mohana N.; Jayarama Reddy; Abhilash M.R.; Satish S. (2024). Unravelling of cellulolytic fungal consortium from humus soil for efficient lignocellulosic waste degradation. The Microbe, 5, 100183, 1-9.
6. Ilangumaran, G.; & Smith, D. L. (2017). Plant growth-promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. Frontiers in Plant Science, 8, 1768.
7. Iqbal, M.; Iqbal, S.; Khan, M. A.; & Akhtar, N. (2020). Mitigation of salt stress in wheat

by combined application of Trichoderma harzianum and biochar. Pakistan Journal of Agricultural Sciences, 57(2), 491–498.

8. Khan, M. A.; Shirazi, M. U.; Mumtaz, S.; Mujtaba, S. M.; & Islam, E. (2010). Impact of soil salinity on the yield and yield components of rice at different transplanting dates. Pakistan Journal of Botany, 42(5), 3857–3865.
9. Koehler, D. E.; Leonard, R. T.; Vander Wand, W. Y.; Linkinks, A. E. and Lewis, L. N. (1976). Association of latent cellulase activity with plasma membrane from kidney bean abscission zones. Plant Physiol., 38, 324.
10. Kumar, S.; & Sharma, A. K. (2020). Role of Trichoderma spp. as a biocontrol agent of fungal plant pathogens. Journal of Pharmacognosy and Phytochemistry, 9(5), 2046–2053.
11. Mohanavelu, A.; Suneja, P.; & Sharma, A. (2021). Halotolerant microbes: a pathway for saline soil phytoremediation. Frontiers in Microbiology, 12, 1423980.
12. Saadaoui M.; Faize M.; Bonhomme L.; Benyoussef N. O.; Kharrat M.; Chaar H.; Label P.; Venisse J. S. (2023). Assessment of Tunisian Trichoderma Isolates on Wheat Seed Germination, Seedling Growth and Fusarium Seedling Blight Suppression. Microorganisms. 6; 11(6):1512.
13. Sharma, N.; and Sharma, N. (2016). Cellulase production and optimization of process parameters by one factor at a time technique from mutant strain of Bacillus stratoshpericus N12 (M) and its scale up in a stirred tank bioreactor. World Journal of Pharmacy and Pharmaceutical Sciences, 5(2): pp.1459-1477. ISSN 2278 – 4357.
14. Wagner, A. O.; Lackner, N.; Mutschlechner, M.; Prem, E. M.; Markt, R. and Illmer, P. (2018). Biological pretreatment strategies for second-generation lignocellulosic resources to enhance biogas production. Journal of Energies, 11: p.1797.
15. Zhang, F.; Li, Y.; & Chen, X. (2023). Trichoderma species: a new strategy for managing soil salinity. Applied Soil Ecology, 180, 104624.
16. Zhao, X.; & Zhang, F. (2015). Effect of Trichoderma inoculation on composting of agricultural waste: a meta-analysis. Bioresource Technology, 198, 277–282.
17. Zheng, Y.; Zhao, J.; Xu, F. and Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. Journal of progress in energy and combustion science, 42: pp.35–53.