AN IN-VITRO ASSESSMENT OF THE ENZYMATIC POTENTIAL OF FUNGAL ISOLATES FOR THE DECOMPOSITION OF PADDY STRAW

Atul Sharma[1], Heena Choudhary[2], Raj Singh[1*], M.K. Rana[3]

Abstract

The present study was carried out to check and assess the enzymatic potential and decomposition capacity of paddy straw by the fungal consortium *in-vitro.* Burning of paddy straw is observed a big problem in Haryana, which is responsible for air pollution in Haryana and NCR regions. The fungal communities play a dominant role in paddy straw decomposition due to their lignocellulolytic potential and can adapt to adverse conditions of the soil ecosystem. Soil is known to be the excellent habitat for fungi. The lignocellulolytic enzyme helps in the formation of humus and thus increases health and fertility of the soil. Total five fungal forms were isolated from the decomposed paddy straw and identified. These five fungi belong to Deutromycotina (*Aspergillus fumigatus, Aspergillus terreus* and *Fusarium* ssp.) and Zygomycotina (*Mucor* and *Rhizopus*). *In-vitro* testing of fungi was done to produce extracellular enzymes, *viz.* cellulose, pectinase and laccase. The findings suggest the possible ways of utilizing the fungal consortium contributing to the green India revolution and making the country pollution free.

Keywords: *In-vitro*, lingocellulolytic potential, paddy straw, decomposition, extracellular enzymes

[1]Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University) Mullana-Ambala, Haryana 133207, India;
[2*]Department of Biosciences and Biotechnology, Banasthali University, Rajasthan 304022, India
[3]Department of Agriculture, Maharishi Markandeshwar (Deemed to be University) Mullana-Ambala, Haryana 133207, India

***Corresponding Author:** Raj Singh
Email: dr.rajsingh09@gmail.com

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INTRODUCTION

Like numerous other horticulture squanders, paddy straw largely comprises of cellulose, hemi-cellulose and lignin (Bajar *et al*., 2020). Out of these, cellulose is the predominant constituent followed by hemicelluloses and lignin (Charaya, 1985). Cellulose like starch is a polymer of D-glucose, but it differs from starch in having β(1-4) linkages, while starch has ά(1-4) linkages and this difference results in strong hydrogen bonding and gives cellulose its physical strength and rigidity (Beare *et al*., 1993). Lignin, along with hemi-cellulose, encrusts the cellulose chains shaping a boundary which avoids wetting and activity of cellulose degradation (Huang *et al*., 2010 and Immanuel *et al*., 2006). To utilize the lingo-cellulosic components as a source of vitality and other needs, this association should be broken to begin with. Any procedure pointed at effective administration of straw must include taking after steps. a) Releasing of the affiliation between lignin, hemi-cellulose, and cellulose. b) Debasement of cellulose, hemi-cellulose, and lignin. c) Encourage change for the utilization of different debasement items for human welfare. Santo *et al*., (2002) considered that the capacity of parasites to corrupt lignocellulosic materials is due to their exceedingly productive enzymatic framework. Organisms have two sorts of extracellular enzymatic frameworks; the hydrolytic framework, which produces hydrolyses that degrade polysaccharide and an interesting oxidative and extracellular ligninolytic framework, which degrade lignin and opens phenyl rings. Karthikeyan *et al*., (2014) appeared that nearness of lignolytic chemicals may improve the action of microorganisms and it would be without a doubt, beneficial utilizing chosen microorganisms display within the straw actually or those which have appropriate properties for extricating the lignocellulosic bond (Kendrick *et al*., 1962). Potential benefits of utilizing microbial strain in mineralization of straw have as of now been illustrated by several specialists (Mohamed *et al*., 2021; Prescott and Vesterdal, 2021; Singh *et al*.., 2021a). It is in this condition that the analysis of lignocellulolytic potential of different fungi breaking paddy straw gets to be vital. This study focuses on the *in-vitro* enzymatic assessment of fungi, isolated from decomposing paddy straw in the natural environment.

2. Material and Methods

2.1 Identification and Isolation of the fungi:

Isolation and identification of the fungi was done in the, Department of Bio-Sciences and Technology, Maharishi Markandeshwar Deemed to be University, Mullana, Haryana. One gm. of straw was cut into 5mm pieces. Put the cutting of the paddy straw in the 100ml of sterile water and stirred it for 15 min to wash it micro propagule well. Then, 90ml of sterilized distilled water was taken and transferred immediately the suspension (1:100 dilutions) that made with 10ml of additional distilled water. This suspension was further used for the serial dilution 1: 1000, 1: 10,000 and 1: 100,000 respectively. From the last three dilutions of the suspensions 1ml were transferred to each Petri dish set with the addition of the 20ml cooled and sterilized Czapek-Dox agar medium along with the 30ppm rose Bengal medium and streptomycin antibiotic. The Petri dishes were incubated at 25±1 for 5-8 days. After three days the fast-growing fungi appears and identified on the basis of their morphological and cultural characteristics (Subramanian, 1971).

2.2 Assessing the ability of fungi to utilize the fungal cellulose:

To assess the ability of utilizing the cellulose by fungi the method suggested by (Fisher *et al*., 1977) with slight modification was adopted. The test fungus was grown in a conical flask each containing 50ml of media, then with the help of the nylon thread a Whatman No.1 paper disc was suspended in the medium in a sterilized flask. The potato Dextrose Agar medium was taken for inoculation of the fungus for 5 days at 25ºC along with the agar-mycelial disc of 7mm and the un-inoculated flask were served as control. The flasks were put on the incubator at 25ºC for 20 days and checked thereafter. To validate the activity the filter paper was removed from each flask and kept in oven at 80ºC to dry it. For comparison, the initial and final weight was measured, the mean percentage loss was calculated with comparison of the test fungus in context of dry weight to check the portion of cellulolytic activity of the fungal species. These losses show the portion of cellulose actively respired not the converted fungal biomass.

2.3 Cx activity:

The isolated fungus was screened to check the Cx (CMCase) activity by the suggested method by (Hankin and Ariagnostakis, 1977). By adjusting the pH of 6.5 of the medium 15ml was poured into petri dishes and then inoculated the fungus in it. After the 5 days of incubation, 1% solution of aqueous hexadecyl trimethyl ammonium bromide poured over the plate, this shows the precipitation

of the carboxy methyl cellulose by making the clear zone around the colony.

2.4 Polygalacturonase (PG) and Pectin lyase (Pnl = PGTE) activity assessment:

The procedure recommended by (Hankins and Anagnostakis, 1975) was used to access the test fungi for PG and Pnl activity. The medium contains pectin in place of CMC rest was same as CMCase. To detect the PG activity the medium with pH 5.0 was used and for Pnl activity medium with pH of 7.0 was used.

2.5 Xylanase activity:

The xylan solution was prepared by dissolving it in hot water and then added it to the Czapek's medium without adding sucrose and made the final concentration of 5% of total xylan in a medium and then 15ml of aliquots of the medium were poured into the sterile petri dishes inoculated with the test fungi. The experiment was carried out in triplicates and fungus was incubated at 25ºC for 10 days and growth was measured in diameters. The mean radial growth of the fungi on the xylan medium were minus from the growth on the medium without xylan was considered as the index of xylanase activity.

2.6 Lignolytic activity:

By following the test suggested by the (Bavendamn's, 1928) the preliminary screening of the test fungi was done, which was based on the phenomenon that the lignolytic fungi can be detected by their reaction with the tannic acid which was oxidized to leave brown color product. The modified Waksman's agar medium was used the test fungus were inoculated and incubated at 28C for 5-6 days. This was observed with the brown coloration around their colony.

RESULTS AND DISCUSSION

About 11 fungi were isolated and identified from five fungus was evaluated for their enzymatic potential in this study i.e., of the identified fungi, Out these five fungi 3-belong to Deutromycotina (*Aspergillus fumigatus, Aspergillus terreus* and *Fusarium oxysporum*) and 2 Zygomycotina (*Mucor* and *Rhizopus*) (Fig.5). *A. terreus* shows higher lignolytic activity, lower cellulolytic activity with moderate pectin. *A. fumigatus* and *Rhizopus ahrrizus* showed higher pectolytic activity than others with low lignolytic activity. *F. oxysporum, A. terreus, Mucor plumbeus, A. terreus* and *R. ahrrizus* presented the three activities (CMCase, pectolytic and lignolytic). The fungi, *A. fumigatus, F. oxysporum, M. plumbeus* show higher pectolytic activity *A. terreus* shows higher lignolytic activity, lower cellulolytic activity with moderate pectin. *A. fumigatus* and *R. ahrrizus* showed higher pectolytic activity than others with low lignolytic activity. *F. oxysporum, A. terreus, M. plumbeus, A. terreus* and *R. ahrrizus* presented the three activities (CMCase, pectolytic and lignolytic) (Table 1, Fig 2, 3, 4). Singh *et al*.., (2021b) considered the potential of fungi for cellulolytic activities. Agreeing to Macauley and and Thrower *et al*. (1966), the organisms able of utilizing cellulose or pectin are vital essential colonizers. Sharma and Khan (1978) have detailed that a few of the starting colonizers, which empowers them to enter and set up within the new tissue some time recently deterioration begins. Kendrick and Burgers (1962) found pectolytic action to be mindful for beginning colonization. Huang *et al*. (2010) detailed most noteworthy lignolytic and great cellulolytic activities during composting. The comes about recommend the plausibility of utilizing these fungi as an indispensably component of microorganism-based strategies for management of paddy crop residue to control the air pollution in NCR regions and enhanced the soil fertility in Haryana and Punjab.

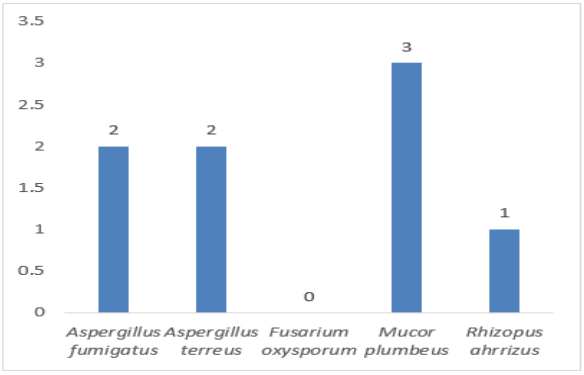

Figure 2: Lignolytic activity fungi.

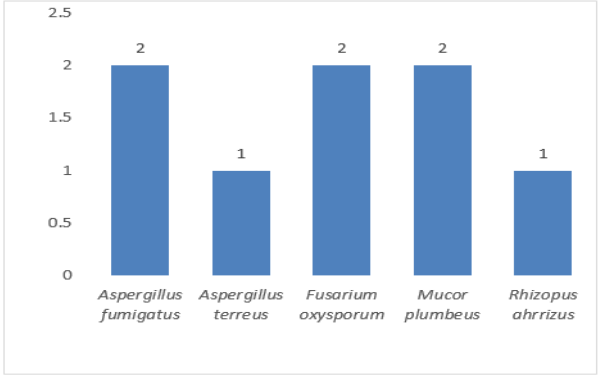

Figure 3: Cellulolytic CMCase activity of fungi.

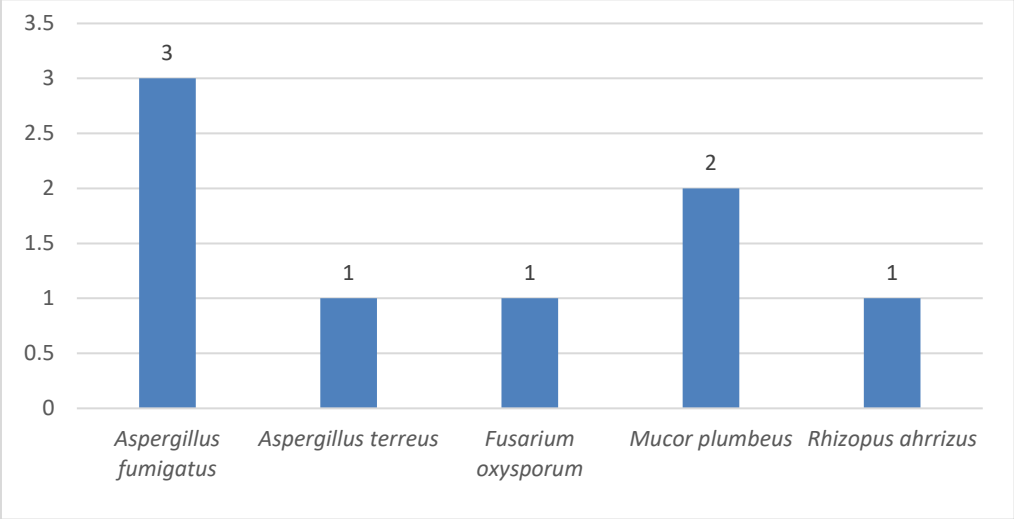

Figure 4: Pectolytic (PG) activity of fungi.

Table 1: In-vitro cellulolytic, hemicellulolytic, pectolytic and lignolytic capabilities of the test fungus.

	Enzyme Activity				
Fungal species	**C_1****	**Cx***	**Xylanase****	**Lignolytic***	**PG***
Aspergillus fumigatus	76	+	82	–	+++
Aspergillus terreus	88	+++	96	++	+
Fusarium oxysporum	91	++	71	–	+
Mucor plumbeus	–	+	86	+++	++
Rhizopus ahrrizus	95	++	78	+	+

*In terms of ranking as compared to control (qualitative test), ** In terms of mean percentage loss of substrate as compared to control.

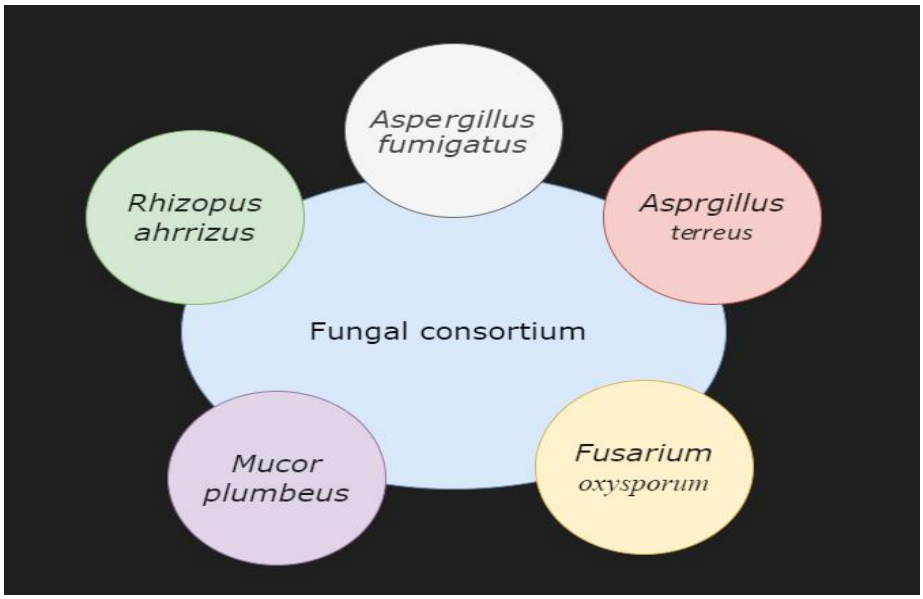

Fig 5: Lignocellulolytic fungal consortium isolated from paddy straw.

CONCLUSION

All agricultural waste consists of lignin, cellulose, hemicellulose, and pectin as well as structural components. The soil inhabited by mycobiota can break down these wastes into their components and break down complex compounds into their simple forms; and nutrients move along the soil profile. This mycobiome-mediated biodegradation of crop residues played a key role in humus formation in the soil, thereby increasing oil fertility and quality. Soil fertility has a direct impact on healthy food production and sustainable agriculture, which improves the country's economy, human and animal health, and the environment. Therefore, this study achieved the main research objective of identifying different

fungal enzymatic potential and their role in the decomposition of paddy straw for further use as organic fertilizer.

Conflict of interest

The author's claims there are no conflicts of interest.

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