

## Nature and activities of microfungi associated with the decomposition of rice straw in Sri Lanka

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### Abstract

Agricultural sustainability through crop residue biodegradation is an eco-friendly method to enrich soil fertility essential to agricultural countries. Rice straw, rich in cellulose, is the primary source of organic matter, enhancing the fertility in rice fields and is a better alternative to replace chemical fertilizer usage. Therefore, this attempt is to isolate and identify different genera of straw degrading microfungi efficient in lignocellulose biodegradation. Rice straw degrading fungal species were isolated from the partially degraded rice straw collected from selected areas in Sri Lanka, following the washing and plating techniques. They were identified into the genus level using standard identification keys, and their capacity to degrade cellulose, starch, lignin, and pectin were evaluated using substrate-specific testing protocols. Eighteen fungal species in the genera of *Aspergillus*, *Chaetomium*, *Cunninghamella*, *Goidanichiella*, *Penicillium*, *Rhizomucor*, *Rhizopus*, *Stachybotrys*, and *Trichoderma* were isolated from the partially degraded rice straw collected from different areas of Sri Lanka. Three *Trichoderma* species showed significantly the highest frequency of occurrences (40%, 45%, and 43% respectively) in rice straw and a homogenous distribution among the collected samples. Furthermore, they were significantly efficient in degrading cellulose, starch, pectin, and lignin. Since the management of crop residues has become an essential aspect of sustaining long-term fertility in cropping systems, incorporation of rice straw, which is rich with cellulose, and the application of *Trichoderma* species into the harvested rice fields will improve the nutrient availability and hence rice yield.

**Keywords:** Degradation, lignocellulose, rice straw, substrate utilization, *Trichoderma* spp.

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### Introduction

Interest in crop residue management and its influence on organic matter and nutrient cycling in soil have been increasing day by day due to the current demand

for agricultural sustainability. Among many crop residues, rice straw is special in Sri Lanka as the most abundant agricultural byproduct rich with cellulose. Applying this main source of organic matter into the rice fields will enhance the fertility of rice fields and



will be a better alternative to replace the chemical fertilizer usage in rice cultivation. However, the management of rice residue through the direct incorporation of straw into soil causes specific problems because of the slow decomposition and weak immobilization of plant nutrients due to its high cellulose, hemicellulose, silica, and lignin contents which make it difficult to degrade (Kumar et al., 2008; Schimpf et al., 2013; Li et al., 2017). Therefore, the application of partially decomposed rice straw is more suitable for maintaining sustainability in the rice fields than non-degraded raw rice straw (Goyal and Sindhu, 2011).

Microorganisms belonging to the groups of bacteria, fungi and actinomycetes are able to accelerate the decomposition rate of rice straw. Different microorganisms show different roles and capacities in the decomposition process as some microorganisms, including fungi, are able to degrade lignocellulose, and some can only degrade cellulose (Andlar et al., 2018). This variation depends on the enzyme production ability in their metabolism. Since cellulose and lignin are complex polysaccharides, only a few microorganisms have the potential in degrading such polymers into simple monomers, and an array of enzymes are responsible for this conversion. The enzymes which can break lignocellulose into stable compounds are called lignocellulolytic enzymes (Huai-Liang, 2010; Mohammad et al., 2013; Kumar and Chandra, 2020). Among microorganisms, fungi are the most efficient microorganisms that are able to carry out lignocellulolytic degradation of rice straw. The results of recent findings revealed that *Fusarium* sp., *Aspergillus terreus*, *Paecilomyces fuisporous*, *Micromonospora* sp., and *Coriolus versicolor* sp. have higher lignocellulolytic activities compared to the other tested microorganisms (Abdulla and El-Shatourym, 2007; Goyal and Sindhu, 2011; Phutela and Sahni, 2012; Chen et al., 2019). In the present investigation, an attempt was given to isolate and identify rice straw degrading microfungi from the partially decomposed rice straw collected from the paddy fields in different Sri Lankan areas and then investigate their enzyme versatility in degrading pure substrates of lignin, cellulose, starch, and pectin. It was also hypothesized that the fungi isolated in a higher frequency of occurrences are efficient in depolymerizing pure substrates of cellulose and lignin, hence opening avenues for the possible application of those fungal genera to accelerate the decomposition of cellulose-rich rice straw.

## Material and Methods

### Sample collection

Partially decomposed rice straw samples (3.5 months after harvesting) were collected randomly from the paddy fields in Gampaha, Kurunegala, Anuradhapura, Kandy, and Hambanthota areas in Sri Lanka during the rainy season following the randomizing block design. Five random replicates were taken from the paddy fields in each area, and then they were transported to the University in sealed sterilized zip lock bags.

### Determination of the washing efficiency of partially degraded rice straw

Twenty pieces (1cm) were obtained randomly from each of the five replicate samples collected from each area with the help of a sharp blade. Then they were transferred into sterilized screw-capped bottles (100 ml capacity) containing 50 ml sterilized distilled water and shaken at 300 rpm/min for two minutes. The water was then decanted into another series of sterilized bottles, and the process was repeated 30 times. One mL aliquot from the above water in each bottle was transferred to tubes containing molten agar (2 % malt extract agar) at 40 °C, agitated by hand, poured into sterilized petri dishes, and incubated for four days at 30 °C (room temperature). Developed fungal colonies in the petri dishes were counted, and the relationship between the number of washings and the number of fungal colonies from each washing was evaluated.

### Isolation of fungi

Fungal isolations were carried out following the modified Harley and Waid (1955) washing and plating technique. One gram of rice straw from each replicate sample was taken, and then it was cut into 1 cm pieces. Randomly selected twenty pieces (1cm) from each replicate sample were transferred separately into 50 ml sterilized water in 100 ml screw-cap bottle and shaken at 300 rpm/min for 2 min using the shaker (Gyrotory shaker, model G2). This washing procedure was repeated eighteen times (as determined previously) to remove surface contaminants. Then excess water in the straw particles was wiped out using sterilized filter papers. Small pieces (1 mm x 1mm) of washed straw samples were plated on potato dextrose agar with streptomycin (0.2 g/L) and tetracycline (0.05g/L). Thirty pieces of straw from each of the five replicate samples collected from each area were plated and incubated at 28 ± 2°C for 5-7 days. Growing fungi in the plated straw particles were isolated into pure cultures using a dissecting microscope (Olympus



313368).

### Identification of isolated fungi

Colony morphological characteristics such as the color, shape, pigment production, and diameter of the isolated fungi were investigated by growing them in PDA (Potato dextrose agar) and 2% MEA (Malt extract agar). The microscopic observations were done using the fungal specimens prepared following the sticky tape method (Flegel, 1980). Using all the characteristic features studied, they were identified into the genus level following online identification keys and other standard identification keys (Domsch et al., 1980; Barron, 1983). A further attempt was given to identify the isolated three species of *Trichoderma* into species level by comparing them with the available identified cultures of *Trichoderma* species (*Trichoderma viride*: AF218788.1, *Trichoderma asperellum*: KT588246.1, *Trichoderma harzianum*: KT852821.1, *Trichoderma longibrachiatum* KP132794.1, and *Trichoderma virens*: KP985643.1) in the Department of Plant and Molecular Biology, University of Kelaniya.

### Determination of frequency of occurrence of each fungus isolated from rice straw

The percentage frequency of occurrence of fungi isolated from the rice straw particles of each of the replicate samples was calculated using the following equation.

$$\text{Frequency of occurrence (\%)} = \frac{\text{No of particles with the specific fungus}}{\text{Total no. of particles plated}} \times 100$$

(Visser and Parkinson, 1975; Kannangara et al., 2001; Kannangara and Deshappriya, 2005)

### Screening of substrate utilization capabilities of the isolated fungi

The potential decomposer abilities and metabolic capabilities of individual fungi of partially decomposed rice straw were tested using the following pure substrates; starch, pectin, cellulose, and lignin (which represent plants' storage and structural components). The ability of the individual fungal species to utilize the above mentioned pure substrates was observed separately according to the procedures given below.

### Cellulose

Cellulose agar medium was prepared with the addition of 1% cellulose powder into the basal medium of Eggins and Pugh (1961). These medium added test tubes were then inoculated separately with the isolated fungi and incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ) for a month or more until the cellulose utilization of the tested fungal species was visualized as clear zones.

### Starch

As described by Kannangara (2002) and Kannangara et al., (2009) agar plates with 5% starch were prepared. The plates inoculated separately with the isolated fungi were incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ) for five days. Then they were flooded with 1%  $\text{KI}_2$  solution, and the amylolytic activity was investigated by the production of clear zones around the fungal colonies.

### Pectin

Polygalacturanase and pectate lyase enzyme activities of the isolated fungal species were assessed using the modified basal medium of Eggins and Pugh (1961), as indicated in Kannangara et al. (2009). Two sets of plates were prepared separately to check two enzyme activities by changing the pH to pH 5 (polygalacturanase activity) and pH 7 (pectate lyase activity). After a 5-7 days incubation period at  $22^\circ\text{C}$ , all the plates were flooded with 1% cetavlon solution (Cetyl trimethyl ammonium bromide). Pectin utilization was indicated by the formation of clear zones around the active colonies.

### Lignin

The fungal species were tested for the production of three enzymes, laccases, peroxidase, and tyrosinase, involved in the depolymerization of lignin by growing them separately on 2% malt extract agar at pH 7 (Kannangara et al., 2009). After a 5-7 days incubation period at  $22^\circ\text{C}$ , drop tests were carried out to observe the production of each of the three enzymes responsible for the lignin breakdown in plant tissue, as indicated in Kannangara et al. (2009).

### Statistical analysis

Data analysis was done using SPSS software package version 17.0. The method introduced by Snedecor and Cochran (1980) was used to analyze the data. The level of significance was set at  $P < 0.05$ .



## Results and Discussion

### Fungal isolation

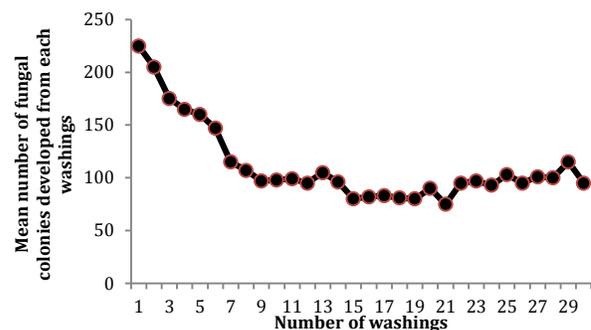
Figure. 1 indicates the results obtained from the experiments conducted to investigate the washing efficiency of partially decomposed rice straw. The detachable fungal fragments that develop into a mycelium rapidly declined from the first washing sample to the seventh washing sample. Eventually, the partially degraded straw samples were gradually cleaned until a low, fairly constant number was displaced in the fifteenth to twenty-first washings. After the twenty-first washing, recolonization ability of the number of the detachable fungal fragment was gradually increased. Therefore, eighteenth washing was taken as the optimum number of washing required to eliminate the surface contaminants from the partially decomposed rice straw.

Serial washing of straw material in sterile water before plating was required to eliminate the surface contaminants of the rice straw. The results of Harley and Waid (1955) revealed that washing techniques give a chance to isolate diverse surface fungal flora of spore derived fungi, mycelium derived fungi, and sterile fungi compared to the diluted plate method and plating unwashed fragments. A minimum number of washings required to eliminate the surface contaminants is called washing efficiency. When the materials were serially washed, surface contaminants (detachable fungal fragments) were gradually removed from the litter. However, if it was done indefinitely, tissue could be damaged, and active fungal fragments colonizing inside the tissue could come out, and they would be removed with decanting water. Therefore, the straw material should be gradually cleaned until a low fairly constant number of fungal colonies are displaced. A fairly constant number of colonies were displaced in the present study for fifteen to twenty first washing. After the twenty first washing, the number of fungal colonies increases again due to the detachment of active fungal fragments inside the tissue of rice straw.

### Frequency of occurrences of isolated fungal species

Eighteen fungal species belonging to the genera of *Aspergillus*, *Chaetomium*, *Cunninghamella*, *Goidanichiella*, *Penicillium*, *Rhizomucor*, *Rhizopus*, *Stachybotrys*, and *Trichoderma* were isolated from the partially decomposed rice straw which was aged for three and half months. Out of these isolates, *Trichoderma* species (*T. viride*, *T. harzianum*, and

*T. asperellum*) showed significantly the highest (40 %, 45%, and 43% respectively), frequency of occurrences in partially decomposed rice straw. Out of other isolated fungal species, *Cunningamela* sp., *Penicillium* sp. 1, *Penicillium* sp.2, and *Goidanichiella* sp. had a comparatively higher frequency of occurrences as 30 %, 23 %, 13%, and 13%, respectively. *Chaetomium* sp.2, *Stachybotrys* sp. and dark sterile sp. showed the lowest frequency of occurrences (3%, 2%, and 2%, respectively) in the decomposed rice straw. All these results revealed that *T. viride*, *T. harzianum*, *T. asperellum*, *Cunningamela* sp., *Penicillium* sp. 1, *Penicillium* sp.2, and *Goidanichiella* sp. were the dominant fungal species involved in the decomposition of rice straw in the paddy fields in the selected areas in Sri Lanka (Figure. 2).



**Figure-1: Mean number of fungal colonies washed off from partially decomposed rice straw shaken in aliquots of sterile water. (Each data point represents the mean of five replicates)**

As per the results of Sangjoon et al. (2011), the fungal species (*A. versicolor* KUC5201, *A. ochraceus* KUC5204, *Aspergillus niger* KUC5183, *Trichoderma harzianum* 1 KUC5182, *Mucor circinelloides* KUC6014, and an unknown basidiomycetes species, KUC8721) isolated from the post-harvest rice straw collected from the eleven different sites in Korea have shown a higher frequency of occurrences. *Trichoderma harzianum* 1 KUC5182 was identified as the best cellulose degrader with the highest frequency of occurrence. Robinson (1994) reported the occurrence of *Trichoderma* spp. at higher frequencies in wheat and corn residues. *Aspergillus* sp. and *Chaetomium* sp. used in the composting process in Japan were isolated at higher frequencies of occurrences from composting paddy straw (Cahyani et al., 2004). Moreover, *Humicola* sp. (Th10), *Aspergillus nidulans* (Th4), and *Scytalidium*

*thermophilum* (Th5), were isolated at a higher frequency of occurrences from the wheat straw samples collected from Indian Agricultural Research Institute (IARI) Farm, yard manure, and soil (Kumar et al., 2008).

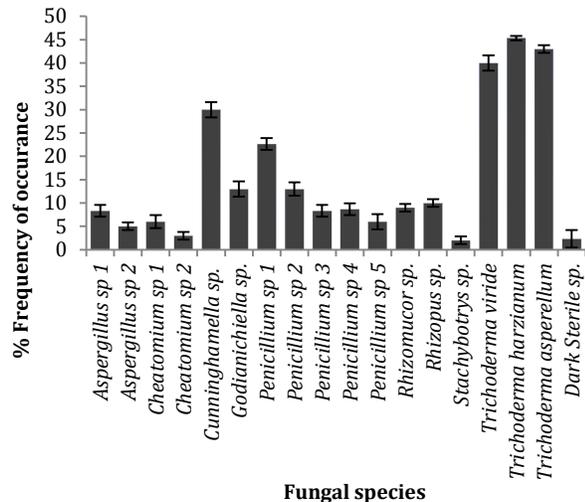


Figure-2: Percentage frequency of occurrences of the fungal species isolated from partially decomposed rice straw (n=5).

### Distribution patterns of fungi in decomposing rice straw

Figure. 3 shows the distribution patterns of each fungus in the decomposed straw samples collected from paddy fields of different areas in Sri Lanka. All the isolated three *Trichoderma* species showed a homogeneous distribution in all the collected samples indicating their abundance at higher frequencies within the country. *Stachybotrys* sp. and dark sterile sp. had the lowest frequency of occurrences, showed heterogeneous distribution patterns in the collected decomposed rice straw samples. In agreement with Sangjoon et al. (2011), the results of the present investigation revealed a correlation between the frequency of occurrence and the distribution of the fungal species in partially decomposed rice straw collected from different areas of Sri Lanka at  $p < 0.05$ . The investigation carried out by Sangjoon et al. (2011), showed significantly higher occurrences of *Aspergillus niger*, *Alternaria alternata*, *Dothideomycete* sp., *Nigrospora oryzae*, *Curvularia intermedia*, and *Cladosporium* sp., almost in all the samples collected from the selected areas in Korea.

### Substrate utilization capabilities of the isolated fungi

Fungi are able to secrete various types of hydrolytic and oxidative enzymes to degrade various polysaccharides such as cellulose, hemicellulose, and pectin (Abd-Elzaher and Fadel, 2010). The results of the investigations on pure non-labile complex carbohydrate utilization capabilities of isolated fungi revealed, all the eighteen fungal species had cellulose degrading capabilities, and only some of them showed the capability in lignin, pectin, and starch degradation (Table. 1).

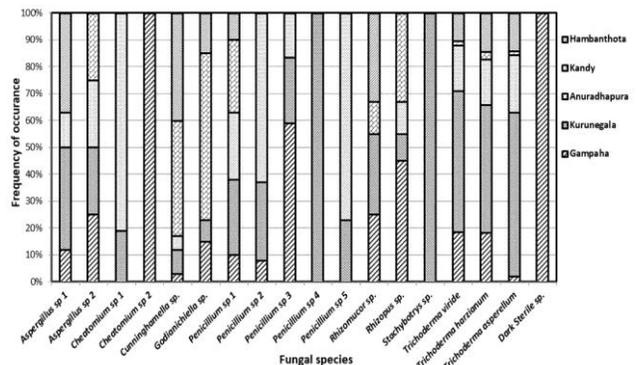
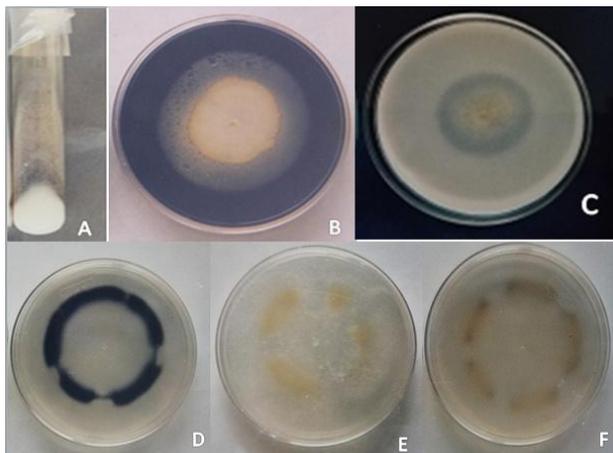


Figure-3: Distribution of the fungal species isolated from partially decomposed rice straw in different areas of Sri Lanka (n=5)

### Utilization of cellulose

*T. harzianum*, *T. viride*, and *T. asperellum*, isolated at the highest frequency of occurrence, were significantly more efficient in degrading cellulose than the other tested fungal species. This was demonstrated by the well-marked clear zones in the test tubes containing cellulose agar (Figure. 4A). These results agreed with the findings of Sangjoon et al. (2011), in which *Trichoderma harzianum* 1 KUC5182 isolated from the post-harvested rice straw collected from eleven different sites in Korea showed the highest cellulolytic and xylanolytic activities. Furthermore, the findings of Bakar et al. (2018), revealed that *Trichoderma* species were able to degrade rice straw due to their high cellulolytic activity. The results of Jorgensen and Olsson (2006); Li et al. (2017) revealed that several *Penicillium* species were able to produce cellulolytic enzymes. The findings of Krogh et al. (2004) showed that twelve filamentous fungi from the genus *Penicillium* were able to produce cellulolytic and xylanolytic enzymes.

All five *Penicillium* species isolated in the present study also indicated the ability to degrade cellulose. Microbial degradation of cellulose is carried out by an array of cellulase enzymes collectively known as endoglucanases, exocellulases, and processive endoglucanases. Gautam et al. (2011), have tested the production of cellulases by *A.niger* and *Trichoderma* sp. during the decomposition process of municipal solid waste, and the results revealed that *Trichoderma* sp. secreted endoglucanase (1.95 U/mL), exoglucanase (1.77 U/mL), and  $\beta$ -glucosidase (1.66 U/mL) in high levels during the municipal waste decomposition compared to *A.niger*.



**Figure-4: Substrate utilization capabilities of the fungi isolated from partially decomposed rice straw**

(A) Cellulase activity of *Trichoderma* sp. visualized as clear zones of hydrolysis, (B) Amylase activity of *Trichoderma viride* visualized as clear zones of hydrolysis, (C) Polygalacturanase activity of *Trichoderma viride* visualized as clear zones of hydrolysis, (D) Diffusion of purplish coloration into the medium indicated the laccase activity of *Trichoderma viride*, (E) Yellow brownish coloration indicated the production of peroxidases by *Trichoderma harzianum*, (F) Diffusion of orange brown colour into the medium indicated the presence of tyrosinase enzyme in the plates containing *Trichoderma harzianum*.

### Starch utilization

In the present study, only ten fungal species (*T. viride*, *T. harzianum*, *T. asperellum*, *Aspergillus* sp.1, *Penicillium* sp. 1, 2, 3, 4, 5, and dark sterile species) were able to decompose starch (Table 1). Fungal enzymes responsible for degrading starch and glycogen are  $\infty$ -amylase, amyloglucosidase,  $\infty$ -

glucosidase, isoamylase, and phosphorylase. Probably  $\infty$ -amylase is the most widely distributed enzyme, and it hydrolyses  $\infty$ -1,4 linkages in the starch polymer (Cooke and Whipps, 1993). The findings of Fioretto et al. (2001) revealed that  $\infty$ -amylase and, to a lesser extent,  $\beta$ -amylase activities occurred at the highest level during the initial stages of decomposition. However, the present findings revealed that three *Trichoderma* spp., *Aspergillus* sp.1, *Penicillium* sp. 1, 2, 3, 4, 5, and dark sterile species were able to utilize starch even though they have been isolated from the three months old rice straw. This was shown by the clear zones around the fungal colonies after flooding with KI/I<sub>2</sub> solution that indicated the amylase activity (Figure. 4B).

### Pectin utilization

Eleven fungal species showed the pectin degradation capability by indicating clear zones on the plates prepared using the basal medium of Eggins and Pugh (1961) and 0.5 % of liquid citrus pectin. Polygalacturonase activity was shown when the medium pH was adjusted to pH 5 (Figure.4C). Pectate lyase activity was shown by *T. viride*, *T. harzianum*, *T. asperellum*, *Aspergillus* sp.1, *Aspergillus* sp.2, *Cunninghamella* sp. *Penicillium* sp.1, *Penicillium* sp.3, *Penicillium* sp.4, *Penicillium* sp.5, and *Rhizomucor* sp. at pH 7. All the three *Trichoderma* species produced significantly larger clear zones indicating their efficiencies in polygalacturonase and pectate lyase activities. According to the findings of Zeni et al. (2011), *Aspergillus niger* ATCC and *Penicillium* sp. isolated from agro-industrial areas were able to produce polygalacturonases in pectin degradation. In agreement with that, in the present study, two *Aspergillus* species and four *Penicillium* species isolated from rice, straw were able to degrade pectin via secreting both polygalatunonase and pectate lyase. However, all the three *Trichoderma* species tested were also able to degrade pectin efficiently with the aid of polygalacturonase and pectate lyase enzymes compared to other isolated fungal species.

### Lignin utilization

Evaluation of lignin utilization potentials of the tested fungi revealed that ten fungal species were able to produce at least one, two, or three enzymes involved in lignin degradation. Out of them, seven fungal species (*Chaetomium* sp.1, *Chaetomium* sp.2, *Penicillium* sp.5, *Stachybotrys* sp., *T. viride*, *T. harzianum* and *T. asperellum*) were able to produce

only laccase and peroxidase (Table.1). However, *T. harzianum* and *T.asperellum* were significantly efficient in producing all the three enzymes laccase (Figure. 4D), peroxidase (Figure. 4E), and tyrosinase (Figure. 4F), indicating their potential in degrading lignin which is a rigid polymer in plants and has the potential for very selective depolymerization. The research findings of Bakar et al. (2018) have also shown the potential in *Trichoderma* spp. to degrade lignin in paddy straw.

**Table-1: Substrate utilization potential of fungi isolated from partially decomposed rice straw**

Fungal species	Cellulose	CMC	Starch	Pectin		Lignin		
				PO	PC	LA	PE	TY
<i>Aspergillus</i> sp.1	+	+	+	+	+	-	-	-
<i>Aspergillus</i> sp.2	+	+	-	+	+	-	-	-
<i>Chaetomium</i> sp.1	+	+	-	-	-	+	+	-
<i>Chaetomium</i> sp.2	+	+	-	-	-	+	+	-
<i>Cunninghamella</i> sp.	+	-	-	+	+	-	-	-
<i>Goidanichiella</i> sp.	+	+	-	-	-	-	-	-
<i>Penicillium</i> sp.1	+	+	+	+	+	-	-	-
<i>Penicillium</i> sp.2	+	+	+	-	-	-	+	-
<i>Penicillium</i> sp.3	+	+	+	+	+	-	+	-
<i>Penicillium</i> sp.4	+	+	+	+	+	-	-	-
<i>Penicillium</i> sp.5	+	+	+	+	+	+	+	-
<i>Rhizomucor</i> sp.	+	-	-	+	+	-	-	-
<i>Rhizopus</i> sp.	+	+	-	-	-	-	+	+
<i>Stachybotrys</i> sp.	+	+	-	-	-	+	+	-
<i>T. viride</i>	+	+	+	+	+	+	+	-
<i>T. harzianum</i>	+	+	+	+	+	+	+	+
<i>Trichoderma asperellum</i>	+	+	+	+	+	+	+	+
Dark sterile sp.	+	+	+	-	-	-	-	-

Even though many researchers have reported the ability of different *Rhizopus* species in degrading specifically pectin (Odeniyi et al., 2009), in the present study, the tested *Rhizopus* species were able to degrade cellulose and lignin only by secreting, peroxidase and tyrosinase. In *Penicillium* spp., the tested two species were able to produce only peroxidases in contrast to Choudhary et al. (2016). They have investigated the

potential in *Penicillium pinophilum* isolated from wheat straw harvested soil to degrade lignin through the laccase activity. In the present study, only *T. harzianum* and *T.asperellum* were able to produce the three enzymes laccase, peroxidase, and tyrosinase necessary to degrade lignin and all the other tested pure substrates.

All in all, out of the eighteen fungal species isolated from the three months aged paddy straw, *Trichoderma* species showed the best cellulases, amylases, laccase, peroxidase, tyrosinase (except *T.viride*) polygalacturonases, and pectate lyases activities compared to the other isolated fungal species. Chemical fertilizer application to rice cultivation in Sri Lanka was carried out widely with high yielding rice varieties for more than four decades. The findings of Yan et al., 2019 revealed that the application of rice straw into the paddy fields at the recommendation rate of four tons per hectare is good enough to provide the total potassium requirement, phosphorus requirement, and 30% of the nitrogen requirement of the rice crop. As per the investigation carried out by Ponnampereuma (1984), rice straw consists of N (0.38-1.01%), P (0.01-0.12%), and K (1.0-3.0%). Unfortunately, most Sri Lankan farmers used to burn or remove the rice straw after the harvesting, and this permanent removal of straw material is an excellent nutrient loss to the paddy fields. This is entirely due to farmers' lack of awareness about the in situ use of rice straw as a nutritional enrichment source in paddy fields (Melissa et al., 2016).

Due to the inconvenience of harrowing and plowing the field in the presence of fresh straw, many farmers in Sri Lanka do not practice the process of recycling rice straw in the field. On the other hand, rice straw decomposition is prolonged due to its high lignin and cellulose contents. This problem could be overcome by accelerating the decomposition of rice straw by introducing indigenous microorganisms efficient in degrading cellulose and lignin into the paddy fields on large scales. However, the research findings of the present investigation highlighted that *Trichoderma* species (*T. viride*, *T. harzianum*, and *T.asperellum*) which were isolated significantly in higher frequencies (40%, 45%, and 43% respectively) from partially decomposed rice straw were efficient in degrading all the pure substrates tested (cellulose, starch, lignin, and pectin) and also were efficient in secreting laccase, peroxidase, and tyrosinase (except *T.viride*) which are the most responsible enzyme in the degradation of lignin. These findings will open the avenues for



possible application of our indigenous *Trichoderma* species into the rice fields in Sri Lanka to overcome the problem associated with the slow decomposition of rice straw and the return of P, K, and N back into the soils.

## Conclusion

Out of the eighteen fungal species isolated from partially decomposed rice straw, *Trichoderma* isolates (*T. harzianum*, *T. asperellum*, and *T. viride*) had the highest frequency of occurrences in the washed rice straw particles. The results of the enzyme activity experiments revealed higher efficiencies of *Trichoderma* species in degrading plant structural and storage compounds. *T. harzianum* was highlighted as the most efficient fungus in utilizing all the pure, tested substrates (cellulose, starch, lignin, and pectin) and were more efficient in secreting ligninolytic enzymes such as laccase, peroxidase, and tyrosinase. Since the higher amounts of cellulose and lignin contents are the causative factors for the slow decomposition of lignocellulosic rice straw, the present investigation indicates the possibility to accelerate the in situ biodegradation of rice straw left in the paddy fields after harvesting through the incorporation of indigenous *Trichoderma* species.

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### Contribution of Authors

Both Undugoda L and Kannangara S equally contributed in this article.

