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Identifying Efficient Isolates of White Rot Fungi for Lignin Degradation of *Calotropis procera* Fibre in Handmade Papermaking

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Abstract: **Presently, there is a huge pressure on papermaking industry to improve its environmental characters. Handmade papermaking is an eco-friendly option. However with the increasing demand of handmade paper, this sector is also facing challenges. *Calotropis procera* is one of the alternative raw materials for making excellent varieties. Owing to its lignocellulosic nature, pulping chemicals are introduced which can be minimized/ eliminated through biopulping with the utilization of white rot fungi. In the present study, white rot fungal isolates identified as *Schizophyllum commune* and *Perenniporia tephropora* have been evaluated for degrading lignin of C. *procera* fibre so that the most efficient isolate may be used further for biopulping to make handmade paper in an eco-friendly manner. *Ganoderma lucidum* (MTCC-1039) was also studied for comparison. To ascertain the lignin degradation capabilities, the development of zones of clearance was observed on the innovatively designed media plates. Accordingly, efficiency of the tested cultures was found to be in the order: *G. lucidum* > *S. commune* > *P. tephropora.* On proximate analysis of the fungal pre-treated fibre, *S .commune* reduced klason lignin to a level of 4.34% from the original value of 12.98% without ill-affecting the cellulose contents. Thus, *S. commune* was identified as an efficient strain for lignin degradation implying its suitability as a biopulping agent in handmade paper industry.**

Index Terms: **Biopulping, *Calotropis procera,* Handmade Paper, Lignin degradation, Paper industry, Proximate Analysis. Swatchh Bharat Mission, White Rot Fungi.**

I. INTRODUCTION

The paper industry, which has been one of the most polluting industries of the world, is recently facing tremendous pressure to improvise economically and environmentally. Since paper is produced conventionally from the woods, there is a strong need of introducing new raw materials and processing techniques to minimize deforestation and pollution levels in papermaking. Due to its tree free nature, handmade paper seems to be very significant in this regard. The history, craft and science of handmade papermaking has been reviewed (Hubbe, 2009).Handmade papermaking is a green and clean industry in the truest sense of its meaning as it uses 100 percent wood free recycled fibres for paper making. Traditionally, the Indian handmade paper manufacturers have been using cotton rags (textile industry waste/denim waste/tailor cuttings etc.) as the principal raw material which is processed in a very simplified manner without the use of harsh chemicals/conditions (Fig.1). The current scenario of "Swatchh Bharat Mission" (Chauhan *et al*. 2016) and ban to 'Single Use Plastic' is boosting the demand of handmade paper and its products **(**Jain *et al*. 2017). With such increase, handmade paper sector is ready to adopt all the modernization techniques and skills. The handmade paper manufacturers are trying their best to compete with the national and international competitors. Competition among different industries is not just for higher production and better quality, but also for better methodology, sustainability and above all, the environmental concerns. The growing popularity and demand of handmade paper has led to sky scrapping prices and scarcity of cotton rags. Therefore, alternative fibrous/lignocelluosic waste materials (which are locally available and easily pulpable viz. Bast fibres/ leaf fibres/ agro-residues etc.) are important to bring sustainability to the handmade paper industry (Jain *et al*. 2013; Mukharjee and Keshri, 2018). However, such materials have to be cooked or pulped using certain chemicals (Fig. 1). Therefore, there is a need to develop eco-friendly pulping and bleaching process techniques so that the eco-friendly credentials of handmade paper industry may be maintained for a long run (Chauhan & Bhatnagar, 2009).

The biotechnological approach namely biopulping may be possibly introduced to minimize such use of pulping chemicals in handmade paper industry. Biological pulping or biopulping is

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nothing but it simply exploits the natural process of wood degradation by extracting/isolating lignin degraders from nature and uses them in a controlled manner for the pulping process of papermaking. Being the most proficient bio-degrader and their capability of degrading lignin selectively in a non-sporulating manner, white rot fungi have been reported to be the appropriate agents of biopulping. With an exhaustive review of its applications, constraints and economical and environmental significance (Singh *et al*. 2010),the process of biopulping that was envisioned as a method for saving energy and making a stronger paper product (Akhtar *et al*. 1998) is reported to transform the pulp production to a basis that is more harmonious to biosphere (Singh *et al*. 2010).

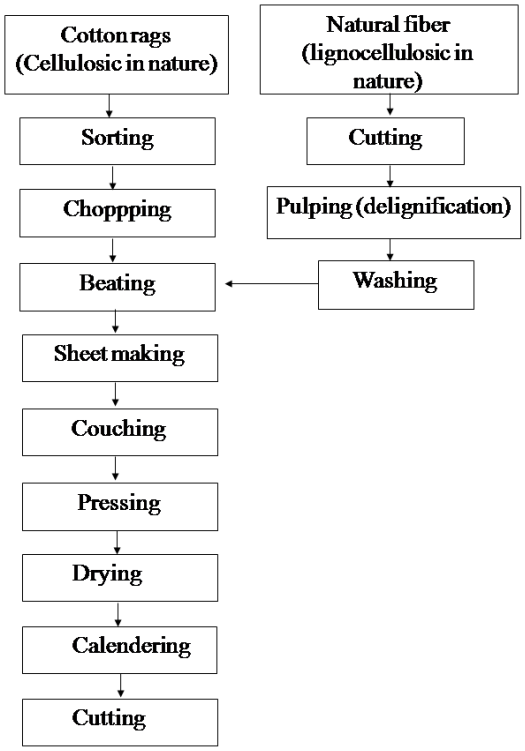

Fig. 1. Process of making handmade paper from cotton rags and natural fibre.

Keeping in view of the above, the present research has been carried out with an aim to isolate efficient strains of white rot fungi from the pink city of Jaipur, and then evaluating the isolates in an innovative manner for their capability of degrading lignin from the fibre of *Calotropis procera*. Based on their lignin degradation capabilities, the selected isolates have been used further for fungal pretreatment of the *Calotropis* fibre to determine their effect on various parameters of the proximate analysis against the control fibre so that the most efficient isolate may be identified for its eventual use in biopulping of *C. procera* to make quality handmade paper in an eco-friendly manner.

II. MATERIALS AND METHODS

A. Isolation and Purification

Potent ligninolytic white rot fungal cultures were isolated from different areas of the Pink city, 'Jaipur'. Potato Dextrose Agar (PDA) was used for isolation. The isolated strains were further screened through qualitative screening methods using chromogenic substrates (Chauhan *et al*. 2017).

B. Identification of the Isolates

Out of the 12 isolates, five isolates showing best qualitative results were further selected for molecular identification. The molecular identification of isolates was carried out through National Fungal Culture Collection of India (NFCC)-ARI, Pune where genomic DNA was isolated in pure form from the isolates and the ITS region of rDNA was successfully amplified using fungal universal primers ITS4 and ITS5. The sequencing PCR was set up with ABI-BigDye® Terminatorv3.1 cycle sequencing kit. The raw sequence obtained from ABI 3100 automated DNA sequencer was manually edited for inconsistency. The sequence data was aligned with publicly available sequences and analyzed to reach identity of the isolates.

C. Culture Conditions

The white rot fungal cultures were grown on PDA media plates at 37°C. Incubation period for the isolate, PSS-2, PSS-5 and *G. lucidum* was 5-7 days while 3-4 days for the isolate, ABC-4.

D. Selection of Raw Material

Calotropis procera Linn. (Vern. Akara, Aak, milkweed etc.) is distributed world-wide but most abundant in the sub tropics growing mostly in dry, sandy and alkaline soils and warm climate. The plant occurs frequently in Indonesia, Malaysia, China and Indian subcontinent as a wasteland weed. This wildly growing stubborn weed is largely available in the sandy tracts of Punjab, Haryana, Rajasthan, Uttar Pradesh and Gujarat states of India besides occurring in Assam and Kanyakumari upto an altitude of 1050m (Meena *et al*. 2011). The inner bark of *C. procera* is used to make strong fibres called madar which are used in the manufacture of weave carpets, ropes, sewing thread and fishing nets (Maji *et al*. 2013). Its stem is a natural source of cellulosic bast fibres having commercially valuable properties like cellulose content, fibre strength, fibre elongation intermediate between cotton and linen, high tensile strength and abrasive strength. It has also been reported to be an excellent alternative raw material for handmade paper making. The *Calotropis procera* fibre is reported to be of very good quality as it contains only 6% lignin and 81.8% holocellulose (Maji *et al*. 2013; Jain *et al*. 2013). The commercially available fibre of *C.procera* used in present study was procured from local market.

E. Dusting of C. procera fibre

The *C. procera* fibre chopped into small pieces of 3- 4 cm was dried at 102 °C. The oven dried fibre was then powdered/dusted by passing through a standard sieve (0.4 mm) in the Dust Making machine. The dust thus prepared was used further for examining the lignin degrading capability of the white rot fungi.

F. Media

Apart from the standard PDA medium used for cultivation of fungal cultures, two types of media (with the nomenclature of AD-Agar Dust and SDA-Sugar Dust Agar) were innovatively designed and prepared as per the composition (AD- 2% *C. procera* fibre dust and 2% Agar, SDA- 2% sucrose in addition to the fibre dust and agar) for observing the zone of clearance produced by the white rot fungi used.

G. Inoculation and Incubation for Evaluating Zones of Clearance

Inoculation of the SDA and AD media plates was done with the freshly grown cultures of the selected isolates i.e. PSS-2 (*S. commune)* and ABC-4 *(P. teprophora)* and the standard culture of white rot fungi *(G. lucidum*) from the PDA plates with the help of Cork Borer. All the experiments were conducted in duplicate. The inoculated plates were incubated at 37°C to see the development of zone of clearance/lignin degradation against the un-inoculated control plates. The presence and intensity of the zone of clearance may actually reflect the lignin degrading capability of the fungus used.

H. Effect of Biological Pretreatment of Calotropis procera Fibre with the Selected Fungal Isolates on Various Parameters of Proximate Analysis

Based on the findings from the evaluation of zones of clearance, further studies were taken up to explore the effect of biological pre-treatment of *C. procera* fibre with the selected isolates on various parameters of proximate analysis as detailed below:

1) Pretreatment of Calotropis procera fibre with selected fungi

C. procera fibre was chopped into pieces of 2-3cm length. The chopped fibres (oven dried weight-75gm) were then filled in the Huffkins flask and autoclaved at 15 psi for 15 minutes. The sterilized flasks were then inoculated with *G. lucidum* and *S. commune* from the freshly prepared PDA plates using cork borer. These two fungi were selected on the basis of their lignin degrading capability observed on SDA and AD plates. Inoculations were actually done with 2 discs of 1mm dia./2.5g of fibre after mixing with the calculated amount of sterilized water so as to maintain a moisture level of 60%. An un-inoculated flask of fibre was used as control under similar conditions. All the treated and control flasks were then incubated at 37°C ±2°C for 15 days.

2) Harvesting of fibres and their preparation for proximate analysis

On 15th day of incubation, the harvested fibres were dried at 102±2°C and grinded. The following parameters were evaluated for proximate analysis of the control and treated fibre as per TAPPI test procedures : Moisture content (TAPPI, T-258 om-02), Weight loss (Kang *et al.* 2007), Ash content (TAPPI, T-211), Silica (TAPPI, T-244), Acetone extractives (TAPPI, T-204), 1% NaOH solubility (TAPPI, T-212), Hot Water Solubility (TAPPI, T-207), Klason lignin (TAPPI, T-222), Acid soluble lignin **(**Schoening *et al.* 1965), Cellulose (Updegroff, 1969), Hemicellulose (Deschatelets and Errest, 1986) and Holocellulose **(**Wise *et al*. 1946).

III. RESULTS

A. Identification of the Isolates

On the basis of 18s RNA molecular identification, the isolate PSS2 was identified as *Schizophyllum commune* and deposited in National Fungal Culture Collection of India (NFCCI)-A National Facility, Agharkar Research Institute, Pune (NFCCI accession no. 4285). The isolate ABC4 was identified as *Perenniporia tephropora* (NFCCI accession no. 4287).

1) Evaluation of the zone of clearance with different isolates used

The normal growth pattern of all the three white rot fungi on the PDA plates can be seen in Fig. 2.However, the growth of these fungi on AD and SDA plates was observed continuously for a period of one month to see the development of any zone of clearance against the un-inoculated control plates of respective media (Fig.3)**.** Zone of clearance was actually a reflection of the lignin degrading ability of the selected cultures in the presence and absence of sucrose. A detailed observation of the mycelial growth (from the front side of the plate) and appearance of zone of clearance (from the back side) at an interval of 5 days was recorded till the 30th day. The specific observations of the zone of clearance with specific cultures were different (Fig. 4, 5, 6).

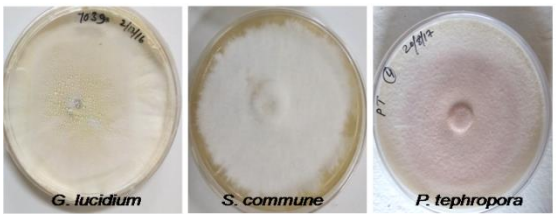

Fig. 2. Fungal cultures grown on PDA plates.

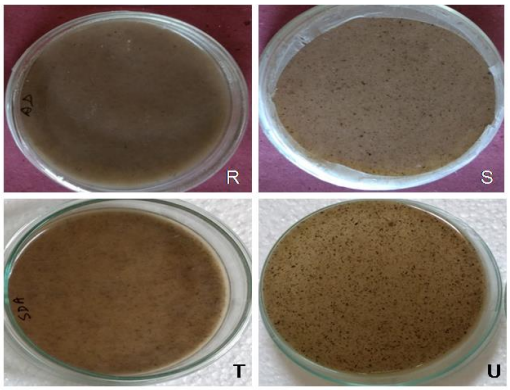

Fig. 3. Un- inoculated Control Plates of AD and SDA
Front (R) and Back (S) view of the AD plate
Front (T) and Back (U) view of the SDA plate.

G. lucidum showed a better white mycelial growth on SDA plates but a better zone of clearance on AD plates. The zone of clearance was developed within 10 days of incubation that became clearer with further incubation (Fig. 4). This implies a better lignin degrading capability of *G. lucidum* in the absence of sugar as compared to its presence.

S. commune showed a denser and cottony mycelial growth on SDA plates as compared to the AD plates. However, the zone of clearance appeared equally on both the plates but in different pattern. The AD plates showed a zone of clearance all over the plate but the SDA plates showed zone of clearance in a streak pattern. Zone of clearance was fully developed in AD plates on 20th day which did not change with further incubation (Fig. 5). Similarly, the streaky pattern of the zone also did not change after 20 days of incubation in the case of SDA plates.

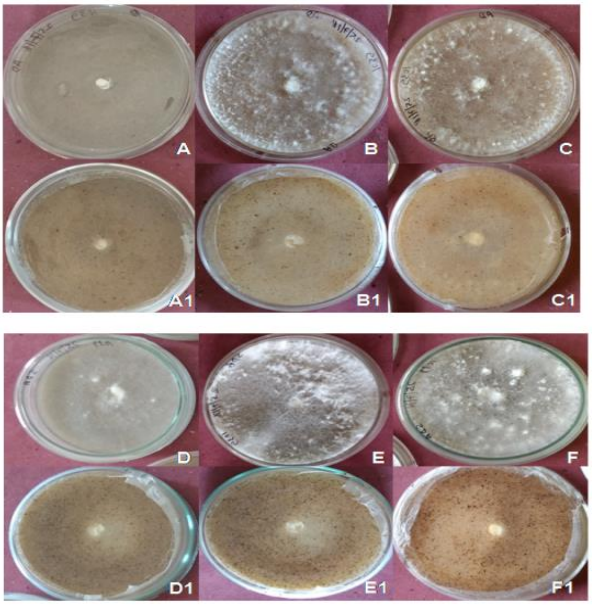

Fig. 4. Pattern of Mycelium Growth (front view) and Zone of Clearance (back view of the same plate) of *Ganoderma lucidum* on AD and SDA Media
AD Plates on 5th (A, A1), 15th (B, B1) and 30th (C, C1) day
SDA plates on 5th (D, D1), 15th (E, E1) and 30th (F, F1) day

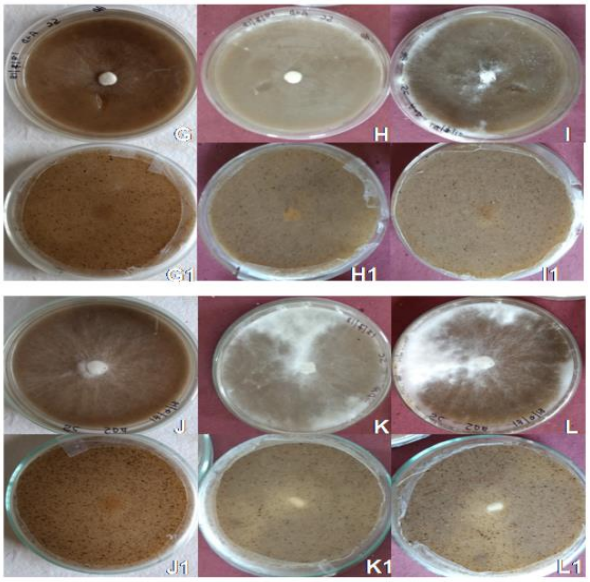

Fig. 5. Pattern of Mycelium Growth (front view) and Zone of Clearance (back view of the same plate) of S.commune on AD and SDA Media
AD Plates on 5th (G, G1), 15th (H, H1) and 30th (I, I1) day
SDA plates on 5th (J, J1), 15th (K, K1) and 30th (L.L1) day

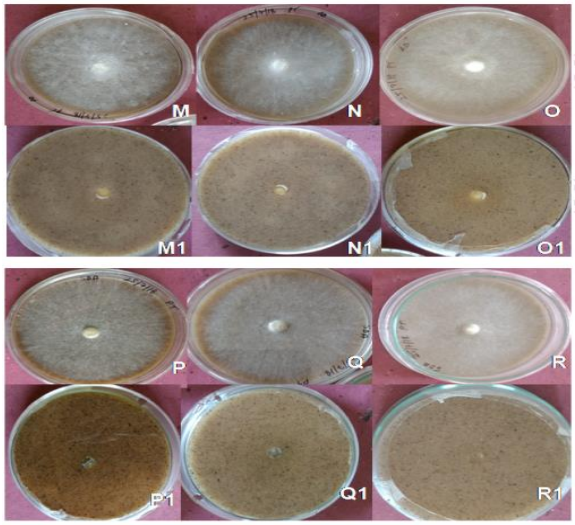

Fig. 6. Pattern of Mycelium Growth (front view) and Zone of Clearance (back view of the same plate) of *Perenniporia tephropora* on AD and SDA Media
AD Plates on 5th (M,M1), 15th (N,N1) and 30th (O,O1) day
SDA plates on 5th (P.P1), 15th (Q,Q1) and 30th (R,R1) day

P. tephropora grew all over the plate within 5 days of incubation. But the zone of clearance could be developed in patches only in the 20th day of incubation. Whereas in SDA plates, zone of clearance did not appear at all thereby showing its inefficacy in lignin degradation (Fig. 6).

From an overall analysis, it could be understood that a clearer zone of clearance was found in the absence of sucrose in all the cultures tested. Degree of the zones of clearance developed

could be indicated in the order *G. lucidum* > *S. commune* > *P. tephropora*.

2) Effect of Biological Pretreatment of Calotropis procera on Proximate Analysis

On the basis of the inferences drawn from the zone of clearance received with different isolates in the presence and absence of sucrose, further studies were conducted with selected cultures under the optimized conditions (i.e. with *G. lucidum* and *S. commune* in the absence of sucrose) with a parallel set of untreated fibre under similar conditions (as a control for reference) to evaluate the impact of fungal pre-treatment on the various parameters of proximate analysis of the control and fungal treated fibre of *C. procera*. One of our isolates, *P. tephropora* did not show any zone of clearance instead it released its own pigment so this isolate was not utilized for the further studies of proximate analysis. Proximate analysis of the treated and control fibres could give an idea about the impact of fungal treatment on the parameters of interest (viz. Cellulose, hemicelluloses, lignin, etc.). Since the production of ligninolytic enzymes is reported to be higher under Solid State Fermentation (SSF) than in the Submerged Fermentation (SF) conditions and SSF is an attractive, robust technology due to its simplicity, cost effectiveness, and maintenance requirements (Rivela *et al*. 2000; Bhatnagar *et al*. 2008), the fungal pretreatment of *Calotropis procera* fibre was carried out under SSF conditions.

Various parameters of proximate analysis evaluated in the control and treated fibres were recorded in Table 1. A graphic representation is given in Fig.7. A close observation of the Table 1 and Fig.7 reflected very important features of high significance. Although the moisture levels were maintained at 60% in all the flasks (having the same lot of *C. procera* fibre with an original moisture content of 5%), yet the moisture values of the treated and control fibres were found to be different on harvesting (i.e. after 2 weeks of incubation). On one hand, control fibre showed a moisture value of 31.13% whereas on the other hand, both the treated fibres showed higher values of moisture and that too was more in the case of fibre treated with *G. lucidum* (41.79±2.8) than that treated with *S. commune* (39.56±4.10). On evaluating the weight loss percentage after harvest of the fungal treated and control fibre, the fibre treated with *G. lucidum* showed a weight loss to the extent of 10.059±4.02 as compared to the fibre treated with *S. commune* which showed a weight loss of only 7.5425±5.27. Although both the treated fibres showed weight loss but the control fibres (C) did not show any. The ash contents were found to be 6.72±0.25, 6.94±0.27 and 9.26±0.35 in control, *G.lucidum* and *S.commune* respectively. On evaluating the silica content, slightly lower values were observed in both the fibres treated with *G.lucidum* (0.55±0.02) and *S.commune* (0.59±0.03) than the control fibre (0.62±0.03). Acetone Extractive is very important parameter of proximate analysis as it is used to remove most water-soluble and volatile compounds that are also soluble in organic solvents. The water-soluble compounds are low-molecular-weight carbohydrates, salts and polyphenols while the solvent extractable material primarily consists of resin and fatty acids and their esters, waxes and un-saponifiable substances. Acetone extractives were found to be lesser in the case of fibre treated with *G. lucidum* (3.85±0.10) and higher in the fibre treated with *S. commune* (5.14±0.15) as compared to the control fibre (4.11±0.12). From the present analysis, values of NaOH solubility were found to be varying from 27.7±1.20 of control fibre to 30.25±1.30 and 31.9±1.45 of the treated fibre samples (*G. lucidum* and *S. commune*) respectively. While analyzing hot water solubility of the control and treated fibres, it was found to vary significantly. It was estimated to be 12.6±0.40 (control), 16.77±0.52 (treated with *G. lucidum*) and 19.14±0.60 (treated with *S. commune*).

Table 1: Proximate Analysis of Control and Treated Fibre.

S.No.	Test Parameter	Untreated Control fibre (%)	Fibre treated with *G.lucidum* (%)	Fibre treated with *S.commune* (%)
1	Moisture	31.13±1.3011	41.79±2.825	39.565±4.104
2	Weight loss	0	10.059±4.028	7.5425±5.271
3	Ash	6.72±0.25	6.94±0.27	9.26+0.35
4	Silica	0.62±0.03	0.55±0.02	0.59±0.03
5	Acetone extractives	4.11±0.12	3.85±0.10	5.14±0.15
6	1% NaOH Solubility	27.7±1.20	30.25±1.30	31.9±1.45
7	Hot Water Solubility	12.6±0.40	16.77+0.52	19.14±0.60
8	Klason lignin	12.98±0.85	6.56±0.40	4.34±0.028
9	Acid soluble lignin	0.08±0.00	0.09±0.00	0.09±0.00
10	Cellulose	70.5±1.65	75.7±1.85	75.2±1.90
11	Hemicelluloses	6.88±0.32	5.92±0.27	7.68±0.35
12	Holocellulose	78.5±1.90	81.9±2.10	82.1±2.20

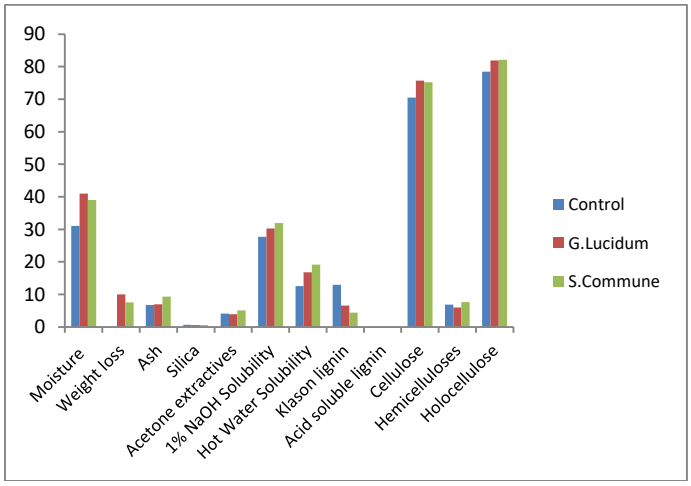

Fig. 7. Comparison of the parameters of proximate analysis for the control and treated fibre of *C .procera*.

Acid insoluble i.e. klason lignin values are important indicators of hardness, bleachability, and the pulp properties like color (TAPPI T-222). Analysis of the control and fungal treated fibre showed significant differences as the obtained values varied from 12.98±0.85 (control fibre) to 6.56±0.40 (fibre treated with *G. lucidum*) and 4.34±0.028 (fibre treated with *S. commune*). On the other hand, acid soluble lignin was found to be slightly higher in the case of treated fibre than the control fibre. As far as the important parameter of cellulose is concerned, it was found to be more in both the treated fibres (75.7±1.85 for *G. lucidum* and 75.2±1.90 for *S. commune*) than the control fibre (70.5±1.65). Similarly, the holocellulose contents were also found to be more in the case of both the treated fibres (81.9±2.10, *G. lucidum* and 82.1±2.20, *S. commune*) as compared to the control fibre (78.5±1.90).

IV. DISCUSSION

A. Evaluation of the zone of clearance with different isolates used

In all the three white rot fungal cultures tested, a better and more prominent zone of clearance was observed in the AD plates than the corresponding SDA plates implying that the lignin degradation was more pronounced in the absence of sucrose rather than in its presence. This finding is in accordance of the earlier reports (Miao *et al*. 2019; Entry *et al*.1993). Entry *et al*. (1993) had studied the effect of nitrogen and carbon sources on lignin and cellulose degradation by *Armillaria*, a white rot fungus and reported that more lignin was degraded as concentration of glucose and fructose increased but not when sucrose concentration increased. In addition to this, a better lignin degrading ability in the absence of sucrose can be further explained in the light of *secondary* metabolism of the white rot fungi. During secondary metabolism, WRF are known to produce ligninolytic enzymes because in the absence of a readily metabolizable source of carbon i.e. sucrose, the fungus will metabolize lignin otherwise the fungi would first metabolize sucrose or any other simple sugar and then attack lignin. Such observations were also recorded by Lee (2005) while studying the application of white rot fungi for biodegradation of Natural Organic Matter in wastes. They have reported lower activities of lignin degrading enzymes in the presence of higher glucose concentrations besides the observation that the fungus didn't attack natural organic matter until the glucose was depleted.

There are also reports showing lignin degrading capability of *G. lucidum* (Hariharan and Nambisan, 2012; Xu *et al.* 2017) and *S. commune* (Irshad and Asghar, 2011; Padhiar *et al*. 2010; Tover-Herrera *et al*. 2018). Although there are reports in literature showing lignin degrading capability of *P. tephropora* especially from the view point of dye decolourization (Ben *et al*. 2007; Ringman *et al*. 2019) but due to the sluggish patch like development of zone of clearance in the AD plates and no zones in the SDA plates besides the pigment formation by the isolate, it was not used for further studies. Due to pigmentation, its media plates became even darker, which is in concurrence of the findings of Ben *et al*. (2007). Thus the AD plates prepared exclusively by the dust of *Calotropis procera* fibre as an exclusive source of carbon and energy with agar powder as a solidifying agent was proved to be very effective for a preliminary identification of the comparative lignin degrading capability of the used fungal cultures.

B. Effect on Various Parameters of Proximate Analysis

With the preliminary idea of the lignin degrading capability of the three fungi in the order of *G. lucidum* > *S. commune* > *P. tephropora*, further studies on proximate analysis of the control and treated fibre were found to be very useful in actually identifying the best fungus for biopulping of *Calotropis procera* fibre because the values of various parameters evaluated were of high significance. Starting from the moisture contents, a little bit higher value in the fibre treated with *G. lucidum* indicates a higher metabolic rate than the *S. commune*. This can be understood in the light of earlier reports (Ringman *et al*. 2019) of natural fungal decays which states that the fungus consumes oxygen and produces carbon dioxide and water when wood carbohydrates are metabolized. As a result, the moisture content in fungal degraded sample is increased. So the fungus actually raises the moisture content to accelerate the decomposition process. Shi *et al*. (2008) have investigated the effects of substrate moisture contents, inorganic salt concentrations, and culture times on the biological pretreatment of cotton stalks using *P. chrysosporium* and reported that the optimum moisture content depends upon the organism and the substrate used for SSF. Isroi *et al*. (2011) have reported that the fungal enzymes viz. laccase, Manganese peroxidase and lignin peroxidases also produce water during catalysis. The terminal electron acceptor in the catalytic reaction of laccase is molecular oxygen, which is reduced to water. Thus the fungal metabolism produces water because of which both the treated fibres showed higher moisture contents.

Similarly, weight loss recorded in the treated fibres as compared to the control fibres indicates the effect of fungal cultures used in degradation of the *C.procera* fibre. Zhou *et al*. (2017) have also reported the use of dry weight losses as one of the important parameters to determine the pre-treatment efficiency of different strains of white rot fungi for wheat straw. Similar reports of weight loss percentage have been made by other workers also (Singh *et al*. 2010; Blanchett *et al*. 1988; Razali, 2015). The higher ash contents of both the treated fibres (*G.lucidum* and *S.commune*) as compared to the control fibre are in concurrence of the earlier findings. Hindi (2017) had reported higher ash in the decayed wood than that of the sound wood.

Similarly, Yalchi and Hajieghrari (2011) have also reported an increase in ash content of the fungal treated fibre as compared to the sound fibre of maize straw, wheat straw, rapeseed straw and soyabean straw.

As far as the important parameter of Acetone Extractives is concerned, Hindi (2017) has reported that higher the acetone extractive, higher is the solubility because of the opening up of anatomical structure of the raw material. However, Kawase (1962) have reported a detailed study of the chemical composition of the wood under three different types of decay showing higher values of alcohol benzene solubility in 'lignin rich' decay whereas not much difference in its values in the case of 'normal-like' decay of wood. In the present study, acetone has been added as an alternative solvent of benzene for the determination of extractives because health, safety and regulatory concerns are associated with the use of benzene and dichloromethane. While studying the natural decay of Hornbeam wood by white rot fungus *Trametes versicolor,* Karim *et al*. (2017) have reported lowering of the contents of alcohol benzene solubility in the decayed wood as compared to the un-decayed wood. Therefore, the values of acetone extractives in the treated fibre found to be higher (in case of *S. commune*) as well as lower (in case of *G. lucidum*) than that of the control may be due to variation in the patterns of metabolism of the individual fungal cultures used.

1% NaOH solubility and hot water solubility are very important parameters for the proximate analysis of any papermaking raw material/pulp because1% NaOH solubility of any sample indicates the degree of fungus decay or of degradation by heat, light, oxidation, etc. As the wood/fibre/pulp decays or degrades, the percentage of the alkali-soluble material increases (TAPPI Test method 212). It also extracts the low-molecular-weight carbohydrates consisting mainly of hemicellulose and degraded cellulose in wood and pulp (Hindi *et al*. 2017).Nearly everyone who has investigated the chemical composition of decayed wood recognizes without exception that the more wood is decayed, the more soluble it becomes in alkaline solution and some of the researchers have tried practically to use the solubility of 1% NaOH as a measure of the degree of wood decay. Thus in the present study, both the treated fibres showing higher values of alkali solubility than the control fibre reflected the impact of fungal treatment on fibre since higher the solubility higher the degree of fungal degradation. Lee *et al*. (2008) have also reported increase in the values of alkali extractives in the treated case as compared to the control ones. In reference to the hot water solubility, there was a noticeable difference among the values obtained for control and treated fibres. Trend in the values obtained was in concurrence with Kawase (1962) who had reported that the decayed wood shows higher values of hot water solubility as both the fibres treated with *G.lucidum* and *S. commune* showed higher water solubility than that of the untreated control fibre. The values of *S. commune* were even higher than those of *G. lucidum* due to the fungal decay of raw material to a greater extent by the fungal isolate.

The obtained values of klason lignin are clear indication of the lignin degradation of *C. procera* fibre by both the fungal cultures. Moreover, the interesting point to note is that our isolate (*S. commune*) was found to be even more effective than the standard culture of white rot fungus used (*G. lucidum*) for lignin degradation thereby indicating it to be a good potent culture for biopulping applications in the handmade paper industry. Lee *et al*. (2008) have reported a loss in lignin percentage ranging from 11.6 to 14.5 during biological pre-treatment of soft wood *Pinus densiflora* by three different white rot fungi. Blanchette *et al*. (1988) and Singh *et al*. (2010) have also reported such decrease/loss in lignin contents in wood quality treated with different white rot fungi. In contrast to the klason lignin values, slightly higher values of acid *soluble* lignin were recorded in the treated cases. Lee *et al*. (2008) have also reported an increase in the values of acid soluble lignin in the soft wood after pretreatment by three different white rot fungi whereas a decrease in the values of klason lignin of the treated materials as compared to the control one.

Any raw material having high amount of cellulose is considered as a good raw material for pulp and paper making. The cellulose contents were found to be much higher in both the treated fibres than the control one indicating the treated fibre as a better raw material than the control fibre. Further, the holocellulose contents were found to be around 82% as compared to the 78% of the control fibre indicating the efficiency of fungal treatment. Yadav *et al*. (2010) have also reported an increase in the contents of holocellulose after fungal pretreatment of the hard wood chips. Similarly, Razali (2015) have reported an increase in the amount of holocellulose after a pre-treatment of the chips of banana pseudo stem with the white rot fungus, *Pycnoporus sangineurs.* Talaeipour *et al*. (2010) have also shown an increase in the cellulose contents during fungal pretreatment of the hornbeam chips. Kawase (1962) have also explained an increase in holocellulose values of the decayed wood (cellulose rich decay) as compared to the sound wood.

Thus the trend of various parameters of proximate analysis of the treated and control fibres was found to be very relevant in ascertaining the lignin degrading capabilities of the tested fungal cultures for the bast fibre of *Calotropis procera.* This is the first report of lignin biodegradation of *C. procera* with the selected cultures of white rot fungi for making ecofriendly handmade papers because most of the earlier investigations have been reported with the woody or non woody raw materials from the view point of conventional papermaking in paper mills. For estimating the economic viability of the process developed, we propose to continue the study with the optimized conditions to eventually process the treated fibres of *Calotropis procera* for making sheets of handmade paper through conventional means

and to compare the results with the sheets developed from untreated control fibre.

CONCLUSION

From the present investigation, it could be seen that out of three fungal cultures used, the standard culture of *G. lucidum* was found to be the most efficient organism for lignin degradation of *C. procera* fibre followed by *S. commune.* Besides, it could be observed that the lignin degradation was found to be better in absence of sucrose rather than in its presence as in the corresponding plate of the respective strain evaluated. The lignin degrading capability of the three strains tested was found as *G. lucidum* > *S. commune* > *P. Tephropora* on the basis of the zone of clearance observed in the respective AD and SDA media plates. Further studies were conducted with the first two fungal cultures for pretreatment of *C. procera* fibre under SSF conditions in the absence of any easily metabolizable sugars viz. sucrose. On evaluation of various parameters of proximate analysis of the control and treated fibres of *C. procera*, the results were quite encouraging from the view point of using both the examined fungal cultures for biopulping applications. The studies conducted have shown that one of our fungal isolates identified as *S. commune* could prove to be a very good candidate for biopulping of *C. procera* because it has degraded lignin in the most efficient manner as reflected from the reduction of its klason lignin values to a level of 4.34% from the original value of 12.98%. The cellulose contents were also found to be more (75.2%) than that of the control fibre (70.5%).

Thus *S. commune* has been found to be the most efficient strain of white rot fungi for lignin degradation of C. *procera* fibre. Its use as a biopulping agent in handmade papermaking from *C. procera* fibre can really revolutionize the industry. The handmade papermaking may play a very important role in providing support to the new entrepreneurs at grass root level thereby helping in making "Atmanirbhar Bharat" to combat the current crisis of COVID'19.

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