

Short Communications

Fungal dry retting — An ecofriendly and water saving technology for retting of jute^a

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Ecofriendly and water saving retting technology of jute has been developed, using pectinolytic fungi by dry fermentation procedure to overcome the shortcomings of conventional retting of jute. Four pectinolytic fungi have been used for these fungal dry retting of jute, viz. *Aspergillus tamarii*, *A. flavus*, *A. niger* and *Sporotrichum thermophile*. This fungal dry retting is found to be an aerobic process unlike conventional water retting, and hence is environment pollution free, faster, water saving and able to produce good quality jute fibre with strong and unbroken full length jute stick as desired by the jute farmers. In field trial, the average fibre strength is 27.7 g tex⁻¹, fibre fineness 2.8 tex and fibre grade between TD-4 and TD-5. These fungi have no adverse effect on the succeeding crop rice. Eight pound regular yarn is prepared from these fibres having normal textile properties. It is expected that this new concept of waterless retting technique by pectinolytic fungi may solve the present water crisis for retting of jute and other allied bast fibres.

Keywords: Dry fermentation, Eco-friendly retting, Fungal dry retting, Jute fibre, Pectinolytic fungus

Worldwide crisis of fresh water for drinking and agriculture has reached to a critical stage in present days due to its non-judicial over use. The natural resources of water, like river, lake, pond, etc. are dwindling very fast. The ground water reserve is also exhausting very quickly due to increased requirement of water for cultivation of high yielding crop varieties. On the other hand, onset of global warming is responsible for irregular climatic condition especially delayed monsoon and scanty rainfall. Extraction of jute fibre from the plants is highly water dependent. In this critical situation any technology to reduce use of water resource has tremendous impact on survival of jute - the highest grown natural lignocellulosic fibre crop.

Jute is a lignocellulosic plant bast fibre mainly grown in Eastern and North Eastern India and

Bangladesh which is restricted geographically between 80°18'E - 92°E and 21°24'N - 26°30'N. However, jute and kenaf (mesta) is also grown in lesser extent in China, Thailand, Indonesia, Nepal, Myanmar and Brazil. Like India, the world's major jute production country, the other jute growing countries are also facing similar crisis of water for extraction of jute by the traditional retting practice. Retting process is conducted by immersing the harvested plants in fresh water where the optimum requirement of water is twenty volume of the plant biomass^{1,2,3}. The fibre and the jute sticks are separated by the joint action of water and retting microorganisms. Retting microorganisms enter from water and adhere to jute plants^{4,5}. Retting of jute in poor quantity of water than its actual requirement degrades the quality of the fibre in terms of its colour, appearance and other physical and chemical properties^{6,7} and consequently lowers its market price.

So, development of a method which requires very little quantity of water for retting purpose is the prime need of the day to help the jute farmers and the jute cultivation. Fungal dry retting of jute using dry jute ribbon was tried^{8,9} in Bangladesh but uniform retting could not be achieved. Present study reports a new water saving and ecofriendly technology for extraction of fibre from jute plants using pectinolytic fungi by dry fermentation technique. This is also a new scientific approach and a breakthrough in retting technology which can help the jute farmers and the cultivation of jute.

Experimental

The fungi used for dry retting of jute were isolated from different retting environments and rotten fruits by enrichment culture technique. Modified Rose Bengal agar medium was used for culturing fungi with jute pectin as main source of carbon keeping other nutrients unchanged. The isolated fungi were purified following standard microbiological procedures and were identified following Manual for identification of fungus¹⁰ as *Aspergillus tamarii*, *Aspergillus flavus* and *Aspergillus niger*. *Sporotrichum thermophile* was collected from Department of Microbiology, University of Delhi. All the fungal species were confirmed by Mycology and

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Plant Pathology Division of Indian Agricultural Research Institute, New Delhi, India. Their specific enzyme activities, viz. exo-pectinase¹¹, pectin lyase¹², xylanase¹³ and cellulase¹⁴ were evaluated following standard procedures.

Fungal Dry Retting Technology

The fungal cultures were maintained on potato dextrose agar (PDA) slants. The above four fungal cultures, viz. *Aspergillus tamarii*, *Aspergillus flavus*, *Aspergillus niger* and *Sporotrichum thermophile* were used for dry retting of jute. Defoliated whole jute plant of different ages, i.e. 90 - 120 days (*Corchorus olitorius* - variety JRO 524) were used for dry retting of jute. pH and E_h before and after fungal retting were determined with the help of a pH meter using combined electrode. Fungal mother cultures were prepared in PD broth. The fungal mats were inoculated on solid base, i.e. a mixture of rice husk and wheat bran following solid state fermentation technique for multiplication. The incubation temperature for *Aspergilli* was kept at 32±1°C and for *Sporotrichum thermophile* at 40±1°C for 7-10 days for complete growth. The fungi grown on solid base were mixed with water in 1:10 ratio and blended with the help of a mechanical stirrer. The suspension containing the fungal growth was sprayed on defoliated green jute whole plants for inoculation. The defoliated jute stem bundles were then covered with polythene sheets and allowed the fungi to propagate on jute plant surface. Polythene sheets were removed after complete fungal growth and fibres were extracted from the stems simply by pulling with hand.

pH in fungal dry retting beds, the moisture regain therein and atmospheric R_H were determined. The extracted fibres were then gently washed in pond water to remove the adhering debris and additional unremoved gum if any and then dried in air. Strength, fineness and fibre grade were determined following standard procedures adopted at NIRJAFT. Standard yarn (8 lbs) was prepared from these fibres and yarn parameters, viz. tenacity, elongation, twists /inch, Um %, thick thin places, work of rupture, tensile strength at break and hairiness were determined using standard BIS certified NIRJAFT methods.

Results and Discussion

Retting of jute is a process of depectinization where pectinolytic microorganisms have a great role in separation of the fibres and detachment of fibre from the jute stick by consumption of the pectin and

other gummy substances⁴. Table 1 depicts the enzymes secreted by the four isolated fungi. The enzymes detected are exo-pectinase, pectine lyase, xylanase and cellulase in different amounts. Pectinase and xylanase play important role in retting process³. Cellulase enzyme is supposed to be harmful in retting process¹⁵ especially after completion of retting. Generally, pectinase, xylanase and cellulase are associated enzymes found in retting microorganisms and they act on specific substrates sequentially. Pectinase enzymes first attack on pectins. Next xylanase consumes easily decomposable hemicellulose, short chain xylan and softens the jute fibre. Cellulase enzyme becomes active when all easily decomposable carbohydrates are consumed. At this stage fibre cellulose is attacked by cellulase enzyme which happens during over retting. So, the microorganism having less cellulase enzyme activity will be more effective for retting purpose³. Cellulase enzyme play important role in bio-polishing of cellulosic or lignocellulosic fabrics due to removal of protruding fibres¹⁶ which is desirable there. From the data presented in table 1 it is evident that *Sporotrichum thermophile* produces highest amount of exo-pectinase enzyme in culture broth followed by the fungus *Aspergillus flavus*. Highest amount of pectin lyase enzyme is produced by *Sporotrichum thermophile* in broth followed by the fungus *Aspergillus niger*. Xylanase enzyme produced by all four fungi in broth culture is found almost at par. Cellulase enzyme production activity by all the four fungi in culture has no significant difference. However, overall enzyme production ability of *Sporotrichum thermophile* in broth culture is found to be higher than those of other three isolated fungi.

This fungal retting system is different from conventional water retting because water retting takes place in submerged condition where anaerobic

Table 1—Enzyme profile of isolated fungai

Fungus (code)	Exo-pectinase U/l	Pectin lyase U/l	Xylanase U/l	Cellulase U/l
<i>Aspergillus tamarii</i> (F-1)	22.1	3.8	24.7	27.0
<i>Aspergillus flavus</i> (F-2)	50.8	8.5	27.2	25.5
<i>Aspergillus niger</i> (F-4)	40.7	10.0	26.8	27.0
<i>Sporotrichum thermophile</i> (S-1)	57.6	15.8	28.5	23.8

condition prevails¹. In contrary fungal retting is conducted in dry and aerobic condition. Generally, retting of jute is conducted during the months between July and August when average day temperature remains around 35°C in most jute growing places. From previous observations, it is found that the optimum temperature for retting of jute is 34°C (ref. 17). All three fungi of *Aspergillus* species have optimum growth temperature at around 32°C. *Sporotrichum thermophile* has a different optimum growth temperature of 45°C. However, this fungus is able to grow at wider range of temperature, i.e. 30° - 50°C. If retting has to be conducted at higher temperature *Sporotrichum thermophile* will be more effective.

The variation in pH and E_h due to the fungal growth in culture broth and in jute retting beds is presented in Table 2. It is evident that all the fungi produce alkaline reaction in culture broth as well as in dry retting beds. The alkaline reaction is found more intense when these fungi grow on jute plants. This finding indicates that the mechanism of dry retting is quite different from water retting¹ and this need to be investigated. Pectin is known to be a polygalacturonic acid with varying degree of branched methyl esters¹⁸. So during decomposition of polygalacturonic acid, galacturonic acid is produced which makes the retting environment acidic under normal water retting system¹. Another remarkable difference is that during water retting the dissolved oxygen is quickly consumed by the aerobic microflora which makes the water environment anaerobic. In anaerobic environment, anaerobic bacteria takes the lead role and as a matter of fact the environment gets polluted by the release of reduced obnoxious smelling gases^{19,20}, like butyric acid, NH_3 , H_2S , CH_4 , etc. From this point of view, fungal dry retting of jute is much more environment friendly than conventional water retting. The redox potential (E_h) during dry retting also remains in the oxidized range. From preliminary experiment, it is evident that all the four

above mentioned fungi perform better retting than conventional water retting.

Dry retting conducted in bench scale followed by in farmers field indicates superiority of fungal dry retting. Fungal retting is completed in 3-4 days earlier in comparison to water retting and the fibres are brighter in appearance. Bench scale trials are conducted with 90, 110 and 120 days plant for dry retting of jute. Table 3 depicts the fibre evaluation properties, viz. fibre strength, fibre fineness, residual root content in the fibre and the fibre grade. Barky root content in 90 days jute fibres is found within 5% range, whereas it is 8% for *A. flavus* in 110 days old jute fibre and the same trend by all the fungi when plant age is 120 days. The fibre strength is found uniformly good in 90 and 110 days plant except for *A. niger* of 110 days plant. The strength of *A. niger* retted jute fibre is found relatively lower, may be due to its higher cellulase enzyme activity. When jute plants retted by individual fungi considered, it seems that the fibre strength is reduced with age of the plant which is not likely. So, there might be some other factor which needs to be investigated. Fibre becomes coarser with age of the plant, as is the general trend with only exception for *Sporotrichum thermophile* in case of plant age 110 days. Plants remain softer and succulent at tender age and become harder with lesser

Table 2—pH of culture and retted jute plant due to fungal inoculation

Fungus code	pH in culture broth	E_h in culture broth	pH in retted jute plants
F-1	8.34	38.2	9.16
F-2	7.86	26.9	8.74
F-4	7.77	17.01	8.90
S-1	8.25	16.4	9.20

Table 3—Evaluation of fibre quality from of different age jute plants by fungal dry retting

Plant age days	Fungus code	Root content %	Fibre strength gtex ⁻¹	Fibre fineness tex	Fibre grade
90	F-1	5	22.7	2.1	TD-5 (60% up)
	F-2	5	24.4	2.8	TD-4
	F-4	5	22.7	2.7	TD-5 (90% up)
	S-1	5	24.2	3.0	TD-5 (50% up)
110	F-1	5	21.5	3.1	TD-5 (70% up)
	F-2	8	21.9	2.8	TD-4
	F-4	5	19.8	3.0	TD-4
	S-1	5	24.1	2.8	TD-5 (60% up)
120	F-1	8	21.6	3.3	TD-5 (50% up)
	F-2	8	20.8	3.2	TD-5 (30% up)
	F-4	8	16.5	3.0	TD-5
	S-1	8	19.6	3.3	TD-5 (50% up)

moisture content and more lignified with the increase in plant age. So, microbial activity gets slower and as a result the fibres become coarser. Retting of jute is believed to happen as a joint action of physical changes by water and microbial enzyme activity. In fungal dry retting, the lead role is taken by the fungal enzymes for the separation of the fibre with very limited available water. So, there is a need to moisten the plants by spraying water if the plants are getting dried during fungal dry retting, because living organisms including fungi cannot survive without water. The fibre grade depends on several factors and in this case the fibre grade remains between TD-4 and TD-5 but mostly closer to TD-4. By overall comparison, it can be said that fibre grade by *A. flavus* is the best.

In these retting trials the average moisture levels are kept between 33% and 35%, during the time of application of fungal inoculum on the jute plant beds and the corresponding average moisture regain is recorded between 50% and 54%. Then atmospheric R_H is found to be 78% which helps the fungus to grow profusely on jute plants for retting.

Fungal dry retting of jute is conducted in farmers' field with the above-mentioned fungi to evaluate the efficacy of the new retting system. Four different field demonstrations cum farmers training trials have been conducted on fungal dry retting of jute with plant age of approximately 120 days during the year 2013 in three different jute growing districts of West Bengal, India. From experimental results, it is evident that the fungal growth propagated on jute plants and simply the jute fibres can be extracted by the farmers from the fungal dry retted jute plants sitting on land.

The efficacy of fungal dry retting at farmer's field has been compared with conventional water retting. The results are presented in Table 4. From the results, it is evident that on an average most of the fungal dry retting conducted at farmer's field is found to produce better quality fibre in lesser retting time, and the average fibre strength is higher and the fibre is finer. In most of the cases root content in fibre is less and fibre grade is higher except in field IV with *A. flavus*. Since this technology of retting is new to the farmers, they need proper training for producing better quality fibre.

The biosafety measure and possibility of any adverse effect of the fungi on preceding rice crop is also evaluated. No adverse effect of the fungi is found on preceding rice crop and on the skin of the farmers

by handling the fungi. Eight pound standard yarn is produced from all the jute fibre samples obtained from fungal dry retting. From Table 5 it is evident that regular yarn can be produced from all four fungal retted jute fibres. It is also evident from the data considering tenacity, tensile elongation, initial modulus, work of rupture, twist, average relative resistance index, U_m percentage, thick and thin place in the yarn and hairiness studies, that mechanical property in case of retting with *Sporotrichum thermophile* is the best among all four fungi, while performance appearance and applicability are the best for fungus *Aspergillus flavus*.

Hence, from the above fungal dry retting experiments, it is clearly evident that fungal dry

Table 4—Evaluation of jute fibre quality and grade from field trials by fungal dry retting

Plant	Fungal code	Root content %	Fibre strength g tex ⁻¹	Fibre fineness tex	Fibre grade
Control	C (Water retting)	10	22	3.1	TD-5
Field I	F-1	5	19.8	2.8	TD-5 (20% up)
	F-2	8	26.2	2.8	TD-5 (60% up)
	F-4	8	27.7	2.9	TD-5 (30% up)
	S-1	8	25.0	2.9	TD-5 (25% up)
Field II	F-1	10	24.8	2.9	TD-5 (80% up)
	F-2	10	23.9	2.9	TD-5
	F-4	8	20.5	2.9	TD-5 (80% up)
	S-1	8	23.7	3.0	TD-5 (45% up)
Field III	F-1	5	17.5	2.8	TD-5 (10% up)
	F-2	8	20.8	2.8	TD-5 (60% up)
	F-4	8	20.7	2.9	TD-5
	S-1	8	20.6	2.9	TD-5 (70% up)
Field IV	F-1	8	20.3	2.8	TD-5 65% up
	F-2	5	16.9	2.8	TD-6 (40% up)
	F-4	10	17.6	2.9	TD-5
	S-1	5	20.5	3.0	TD-5 (80% up)

Table 5—Yarn character of fungal dry retted jute fibre

Fungal code	Tenacity at maximum load cN/tex	Tensile elongation at break %	Initial modulus cN/tex	Work of rupture mJ/tex m	T.P.I.	Average relative resistance index	Um %	Thick places per km	Thin places per km	Hairiness index %
F-1	9.91(19.49)	2.04(13.08)	202.31(27.54)	0.83(30.44)	4.24(4.91)	2807.72	24.79	2334	1550	13.07
F-2	10.67(23.24)	1.97(18.89)	237.60(31.18)	0.86(37.18)	4.36(6.84)	3654.65	24.46	22.32	1436	11.90
F-4	10.52(20.49)	2.25(13.27)	194.05(21.35)	0.96(30.62)	4.32(7.37)	3573.97	25.72	2596	1564	11.28
S-1	10.60(20.02)	2.48(12.96)	112.75(28.94)	1.00(31.45)	4.10(8.67)	3378.99	27.80	3246	1874	12.48

Figures in parentheses indicate CV% values.

retting of jute is able to reduce water requirement for retting purpose in ecofriendly manner; it produces better quality jute fibre in lesser time; and regular yarn can be produced from these fibres. The advantages of fungal dry retting are as follows:

- Fungal dry retting is an aerobic process, so it does not pollute the environment by producing any offensive smelling gases.
- There is no chance of producing methane gas.
- Fungal retting is faster. It does not allow breeding of mosquitoes.
- Farmers can extract the fibre sitting on dry land instead of standing in dirty polluted water.
- It may be an effective tool for fighting against anthropogenic factor responsible for global warming. Jute farmers can even get carbon credit by adopting this technology.

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