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Evaluation of the effectiveness of water submersion method for protection of bamboo from borer damage

(Final Report of the Project KFRI 557/2008)

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PROJECT PROPOSAL

Project No.	:	KFRI 557/08
Title	:	Evaluation of the effectiveness of water submersion method for protection of bamboo from borer damage
Principal Investigator	:	K.V. Bhat
Associate Investigator	:	M. Balasundaran
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ABSTRACT

Submersion of freshly harvested bamboo culms in water for a certain length of time is a traditional method of bamboo preservation followed in rural areas of India. A previous study has indicated a decrease in starch content of culms as a result of this treatment and the involvement of some microorganisms with starch depletion. The present study was conducted to investigate the rate of starch depletion in culms stored under water and the role of microbial population in the process. It was found that the starch stored in culm tissues was reduced by more than half in a two months period at a slow and gradual pace. Extending the length of submersion period by one more month led to further reduction in starch content. The activity of starch hydrolyzing enzyme α-amylase by microorganisms was responsible for the starch depletion. Even the water used in the submersion experiment showed amylase activity but it was low as compared to that in the tissue extract from submerged culms.

The total microbial population comprising bacteria, fungi and actinomycetes within bamboo tissues increased drastically within 15 days after submersion. While aerobic microorganisms showed an early decline after 15th day, anaerobic organisms capable of starch degradation continued to increase up to 45th day. Increase of aerobic microorganisms in stagnant water was not considerable whereas their population increased in running water. Thus anaerobes were the most active starch degraders in stagnant water whereas, aerobes could degrade starch only in running water where oxygen is continuously replenished. About 75% of the bacteria were gram-positive spore forming *Bacillus* species, while 25% were gram negative cocci. There were a few actinomycetes but fungi were rare. The total anaerobes increased continuously even 60 days after keeping the bamboo pieces in stagnant water. But the population increase for aerobic as well as anaerobic starch degraders was not very rapid. The increasing populations of starch degraders within the submerged bamboo tissues and in bamboo-soaked water explain the decline in starch content when submerged under water. It is thus evident that water submersion treatment leads to depletion of storage starch in bamboo culms due to microbial activity and makes it less attractive to borers.

INTRODUCTION

The damage caused by borer beetles has been the main hurdle preventing wider utilization of bamboos. In order to contain the problem, a variety of traditional and modern protective measures have come into being, although at different points of time. Storage or submersion of bamboo in water soon after felling for a few weeks is one such traditional preservation method used by indigenous communities and farmers of several Asian and Latin American regions. Experience gained through ages has shown that this treatment improves the durability of bamboo as compared to untreated material. In Indonesia, Vietnam and Africa, the most widely followed practice for increasing the durability of bamboo is soaking in water (Sulthoni, 1987). In India, submersion of bamboos has been in practice in rural areas, for centuries, for increasing the service life of constructional bamboo and also to improve the pliability of the material for basketry and mat-weaving. It has been observed that during the soaking period, the starch content of bamboo is reduced (Plank, 1950; Beeson, 1941; Chowdhury, 1993; Hidalgo, 2003), and the material becomes less attractive to borers (Liese, 1980; Tamolang, 1980). Bamboos with depleted carbohydrates become reasonably resistant to the attack of borers. For example, culms from flowered clumps that are depleted of starch after seed setting are found to escape borer damage (Bhat and Varma, 2006). However, the exact reason behind the reduction in starch during the submersion treatment is unclear (Liese, 1980). Although it has been suggested that the treatment leads to leaching out of sugars/starch from bamboo, the exact mechanism of such leaching has not been investigated. Starch being insoluble in water and not readily leachable, the process by which it is rendered soluble to facilitate leaching, needs a plausible explanation.

In an earlier study (Bhat and Varma, 2006) it was found that there was a noticeable reduction in starch content of culms as a result of a month-long submersion of culms in water. Storage in stagnant water, particularly, caused greater depletion of starch, which was attributable to the activity of some saprophytic microorganisms multiplying in the submerged samples. However, starch depletion in samples submerged in running water was slightly lower.

The present study was undertaken to determine experimentally the rate of starch depletion and the underlying biological basis, and to standardize the treatment methodology for optimum results. The objectives of the project were:

1. To determine the extent of starch degradation due to submersion of culms in water
2. To identify the microorganisms involved in starch degradation
3. To standardize the treatment methodology for optimum results

MATERIALS AND METHODS

Culm samples of two common bamboos, *Bambusa bambos* and *Dendrocalamus strictus* were collected from the forest areas of Attappady, Kerala. The basal half of the felled culms was subdivided into 0.75m long segments and tied into bundles for submersion. From each culm, one segment was used for estimation of initial starch and also for culturing the microorganisms initially present in the samples at the time of collection. The bundles of bamboo segments were placed under water filled in two rectangular tubs of appropriate size. Weights were stacked on the bundles to keep the latter submerged. In order to create two experimental conditions namely, running and stagnant water, one of the tubs was provided with a continuous supply of flowing tap water to create a condition similar to running water while the other was left without any extra supply of water. From the experimental set up so established in the open samples were periodically drawn at an interval of two to four weeks for estimation of starch and amylase activity. Amylase activity was determined both from the extract of bamboo tissue and from the water used for submersion experiment. The experimental set up was maintained for three months.

Periodical estimation of starch and amylase activity was conducted at fortnightly intervals in the initial set of experiment. However, as the changes noticed were only minor, in subsequent experiments, the interval for analysis was extended to one month. The method used for the determination of starch and α-amylase is given below.

Estimation of starch

Starch content was estimated using the technique of Humphreys and Kelly (1961). The culm samples from running and stagnant water were first dried, powdered and sieved through a 200 mesh size sieve. The powder was first treated with 7.2M perchloric acid and the reaction was allowed to continue for 10 min with occasional stirring. The contents were then transferred to a 50 ml volumetric flask and brought to volume with distilled water. The solution was centrifuged at 4000 rpm and 10 ml of aliquot was made alkaline with 2N NaOH using phenolphthalein indicator. To the solution, 2N acetic acid was added until the indicator colour discharged and then a further 2.5 ml was added. Then, 5 ml of 10%(w/v) potassium iodide and 5 ml of 0.01N potassium iodate were added. The colour was allowed to develop for 15 minutes and the solution was brought to volume. The absorbance was measured on a spectrophotometer at 620 nm in comparison to a blank prepared without starch as zero. The starch content was estimated with the help of a reference curve plotted using potato starch. The percentage of starch was expressed with respect to gram dry weight of the material.

Determination of α-amylase

Determination of α-amylase activity was done by iodometric method. The enzyme source used was the extract from the bamboo samples and the water used for soaking experiment. Sodium acetate buffer (1 ml) of pH 5 was mixed with 1 ml of 0.5% soluble starch solution. The solution thus prepared was incubated for 10 minutes at 37° C. Then 1 ml diluted culture filtrate/ extract was added as enzyme source. For control samples, distilled water was used in the place of extract. 1 ml of 0.5M HCl was added after 10 minutes to stop the reaction. 0.2% Iodine Potassium Iodide was added and the solution was made up to 50 ml. Colorimetric reading was recorded using 570 nm filter. The starch content was estimated with the help of a reference curve plotted using potato starch and the quantity of starch depleted in mg was converted into units of amylase activity.

Microbial population in bamboo and water

Dilution plate technique was adopted for estimating the microbial population in bamboo tissues and in water. Bamboo samples were chopped into small pieces and ground in a mixer grinder aseptically. Ten grams of the ground sample was aseptically transferred to 90 ml sterile water blank. After shaking thoroughly on a rotary shaker for fifteen minutes, serial dilutions of the solution up to 10^{-4} were prepared using sterile water. Exactly 0.1 ml of the final solution was transferred to Petri dishes containing starch agar medium. The samples were incubated at room temperature for enumeration of aerobic microorganisms and in an anaerobic chamber for enumeration of anaerobic microorganisms. The microorganisms growing in the Petri dishes were enumerated by counting the number of colonies of bacteria, fungi and actinomycetes. The total microbial population expressed as colony forming units per gram (cfu/g) bamboo tissue was calculated.

For enumerating the amylase producing colonies, the starch agar plates were flooded with Lugol's iodine solution which leaves a clear zone around amylase producing organisms due to the enzymatic hydrolysis of starch. The aerobic and anaerobic microbial population present in water before submersion of bamboo pieces (initial population), and 15 days, 45 days and 60 days after submersion were estimated adopting the same method.

RESULTS AND DISCUSSION

The average basic density of mature culms of *B. bambos* and *D. strictus* used in the present study was in the range of 650- 700 kg/m^3. The moisture content was 65 and 76 per cent respectively.

Microscopic examination of freshly felled culm sections of *B. bambos* and *D. strictus* showed dense accumulation of starch in ground parenchyma cells (Fig. 1a). The short cells of ground parenchyma did not contain starch grains. So also the fibro vascular tissue of the culms was devoid of starch. However, culms submerged under stagnant (Fig. 1b) and running water (Fig. 1c) for 2-3 months showed conspicuous reduction in starch content in the parenchyma cells at the end of the storage period.

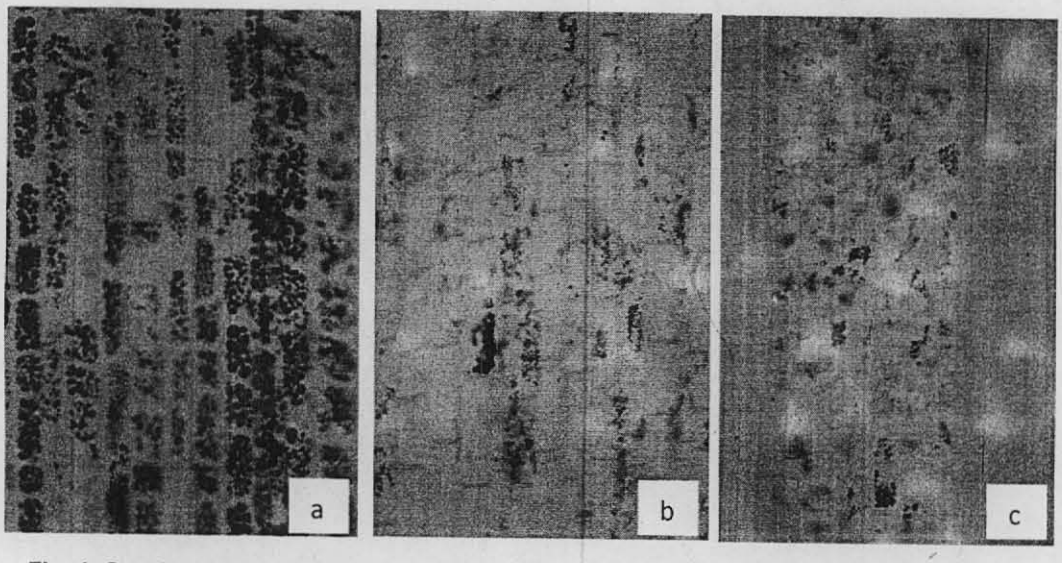

Fig. 1. Starch grains in parenchyma cells stained with iodine-potassium iodide. a.Freshly harvested bamboo. b,c. Bamboo with depleted starch after two months' storage under water

From spectrophotometric analysis in the initial experiment it was found that the starch content of the culms of *B. bambos* and *D. strictus* reduced by more than half of the original at the end of a 2 month submersion period (Table 1). Starch depletion was marginally higher in culm samples submerged in running water in comparison to those placed under stagnant water. However, the stagnant water used was more fouled as compared to running water which indicated the intense activity of saprophytic microorganisms. It has been suggested that when bamboo is treated by water soaking in basins, the water needs to be changed regularly to avoid fouling (http://www.chalet-bamboo.com/treatment.html.).

It was found that even the water used for bamboo submersion experiment, showed the property to degrade starch, obviously due to the enzyme activity of the microbial population present in the water. In order to confirm this observation, the water used for the experiment was used as a source of enzyme and the activity of α- amylase was

Table 1. Decrease in starch content of culm samples subjected to submersion in water

		Percentage of Starch				
		Initial	After 15 days	After 30 days	After 45 days	After 60 days
Bambusa bambos	Running Water	6.3	5.1	4.4	3.6	2.9
	Stagnant Water		5.4	4.5	3.8	3.0
Dendrocalamus strictus	Running Water	6.25	5.8	5.2	3.8	2.70
	Stagnant water		6.1	5.8	4.8	3.75

determined. Distilled water was used as control for comparison. It was found that the extract showed 1.0 to 1.10 units of α-amylase activity.

The decrease in starch content of experimental culm samples, between successive fortnights was gradual and less pronounced. Hence, in subsequent sets of experiment the frequency of quantification was brought down to once in a month and the total duration of the experiment was increased to three months. The experiment was replicated thrice and the averages of values are given in Table 2.

Table 2. Average starch content (%) in submerged bamboo samples

	Bambusa bambos		*Dendrocalamus strictus*	
	Running water	Stagnant water	Running water	Stagnant water
Initial	3.72	3.72	6.33	6.33
After 1 month	2.14	3.03	5.16	5.01
After 2 months	1.89	2.23	3.95	3.75
After 3 months	1.67	1.89	2.71	2.51

It is seen evident from the Table 2. that due to prolonged submersion, the starch content of culm samples of both *B. bambos* and *D. strictus* was further reduced as compared to a 2-month long submersion. Thus it appears to be advantageous to keep the culms under water for a longer time to facilitate greater depletion of starch from them. Earlier studies on water submersion elsewhere have suggested a submersion period of 4 to 12 weeks (Kumar *et al.*, 1994). However, a prolonged submersion is not found ideal in view of the bacterial decomposition of the lignocellulosic material that can happen in course of time.

Activity of α-amylase was determined taking both extract from submerged samples and the water used for submersion experiment as enzyme sources. The average values of three replicates analysed are shown in Table 3.

Table 3. Amylase activity (in units) in water-submerged bamboo samples

Species	Experimental condition	α-Amylase activity (in units)	
		Water as enzyme source	Water soaked bamboo as enzyme source
Bambusa bambos	Running water	0.52	1.41
	Stagnant water	0.59	1.55
Dendrocalamus strictus	Running water	0.61	1.61
	Stagnant water	0.50	0.91

It is seen that the α-amylase activity was generally higher when the extract from submerged samples was used as enzyme source. It is quite expected that the tissue samples in which starch degradation occurs will have a richer population of microbes as compared to the surrounding water medium. There was no consistent difference in enzyme activity between the species or between the two experimental conditions namely, stagnant water and running water.

Microbial population

The total microbial population within bamboo tissues increased drastically within 15 days. In *B. bambos*, the total microbial population comprising bacteria, fungi and actinomycetes further increased till 45th day and then decreased (Figs. 2, 3). While aerobic microorganisms showed an early decline after 15th day, anaerobic organisms continued to increase up to 45th day. This is as expected, because aerobes cannot survive and multiply for a long period since the oxygen gets depleted rapidly by their own growth. However, there could be facultative aerobes and anaerobes among the microbial populations which enhance their adaptability and survival to the changing environmental conditions. The anaerobes were almost completely bacteria, whereas aerobes comprised more than 95% bacteria. The trend of gradual decrease in starch content of the water-submerged samples for three months as in the present study can be attributed to the enzyme activity of the multiplying bacterial population in the tissues and the water. A number of earlier studies (Plank, 1950; Sulthoni, 1987; Kumar *et al.*,1994; Chowdhury, 1993; Hidalgo, 2003) have observed that in water soaking treatment, starch and sugars in culms is reduced which is assumed to be due to leaching out of these substances. However, the process has not been examined in detail and the role of microbial involvement in the process has not been reported.

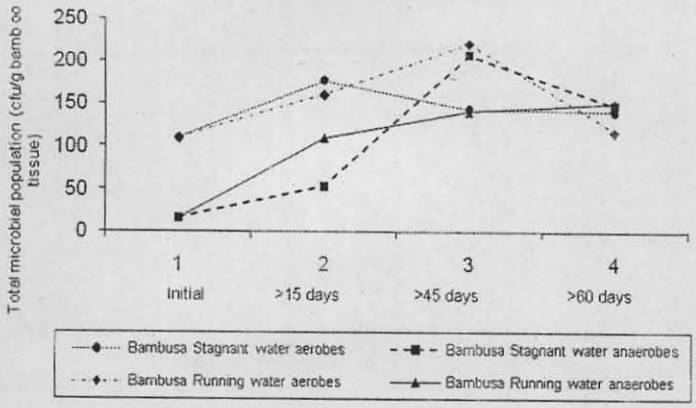

Fig. 2. Total microbial population within *B. bambos* tissues at different intervals of water soaking

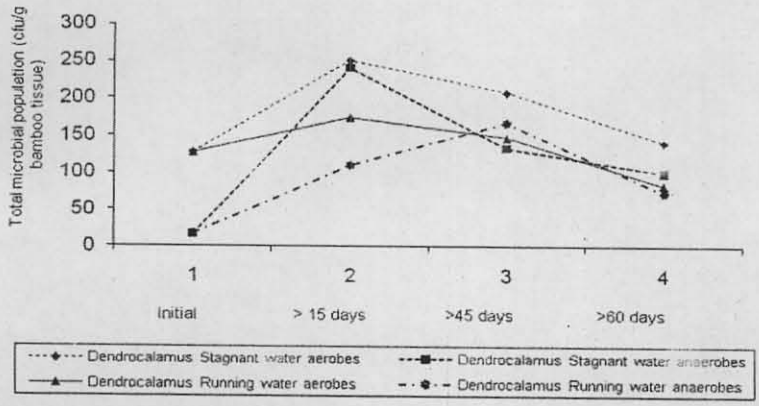

Fig. 3. Total microbial population within *D. strictus* tissues at different intervals of water soaking

The quantity of anaerobic microorganisms capable of starch degradation increased in the bamboo tissues rapidly and reached maximum by 15th day itself. However, the increase in population of aerobic microorganisms in respect of *B. bambos* and

Table 4. Population of microorganisms capable of starch degradation

Bamboo species	Type of sample	Microbial population (x 10^4 cfu per g of bamboo)							
		Initial population		After 15 days		After 45 days		After 60 days	
		Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes
B. bambos	Fresh bamboo	6.7	-	-	-	-	-	-	-
	Stagnant water	-	-	16.6	76.7	16.67	33.39	16.6	6.7
	Running water	-	-	63.3	66.7	26.6	16.6	6.7	16.6
D. strictus	Fresh bamboo	0.03	3.3	-	-	-	-	-	-
	Stagnant water	-	-	6.67	130.3	16.6	16.6	3.3	10.0
	Running water	-	-	73.3	70.0	20.0	46.7	10.0	13.3

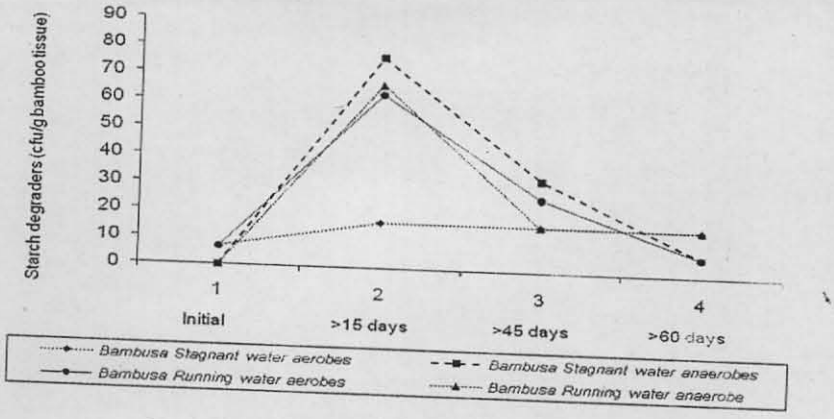

Fig. 4. Quantity of starch degraders among the total microbial population within the tissues of *B. bambos*

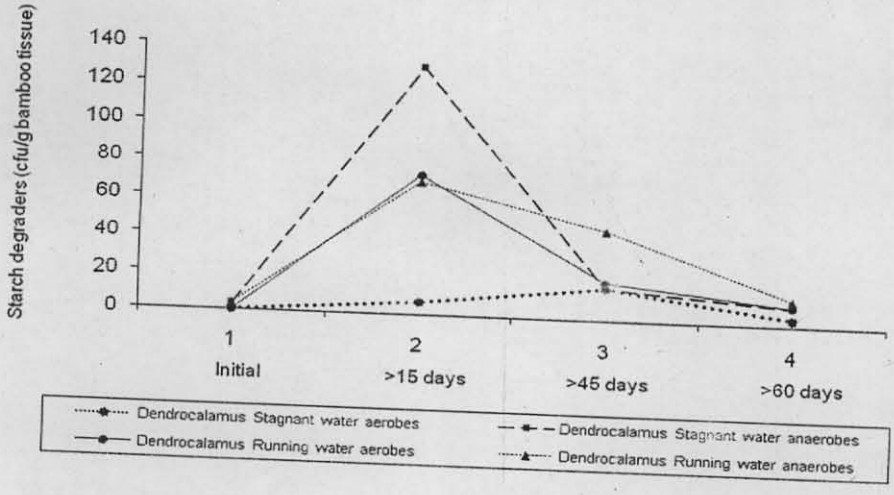

Fig. 5. Quantity of starch degraders among the total microbial population within the tissues of *D. strictus*

increase in samples kept in running water. This indicated that while anaerobes are the most active in stagnant water soaked samples, aerobes can degrade starch only in running water where oxygen is continuously replenished (Table 4; Figs. 4, 5).

Out of the starch degrading organisms, 99% were bacteria. About 75% of them were gram positive, spore forming *Bacillus* species while 25% were gram negative cocci. In addition, there were a few actinomycetes, but fungi were rare.

Table 5 and Fig.6 reveal that the total anaerobes increased continuously even 60 days after keeping the bamboo pieces in stagnant water. But the population increase for aerobic as well as anaerobic starch degraders were not very rapid. Their populations increased approximately by 25 % while the total population increased several fold.

Table 5. Microbial population in water sample

Nature of water	Population of microorganisms (x 10^4) in 1 ml of water			
	Total microbial population		Starch degrading microorganisms	
	Aerobes	Anaerobes	Aerobes	Anaerobes
Fresh water	9.8	-	-	-
Stagnant water after 45 days	173.3	293.3	76.7	100.0
Stagnant water after experiment (after 60 days)	256.7	450.0	113.3	126.7

The increasing populations of starch degraders within bamboo tissues as well as in bamboo-soaked water explain the decline of starch content within the tissues of bamboo when stored under water. Large populations of starch degraders might be producing amylase enzymes not only within the bamboo tissue but also in the water where the samples are soaked. Microorganisms which show large clear zones around microbial colonies in Lugol's iodine-treated culture plates demonstrate their potentiality in scavenging the starch grains available in the bamboo tissues (Fig.7). The amylase activity detected in the water-soaked bamboo tissues and the water used can thus be attributed to the starch degrading bacterial population.

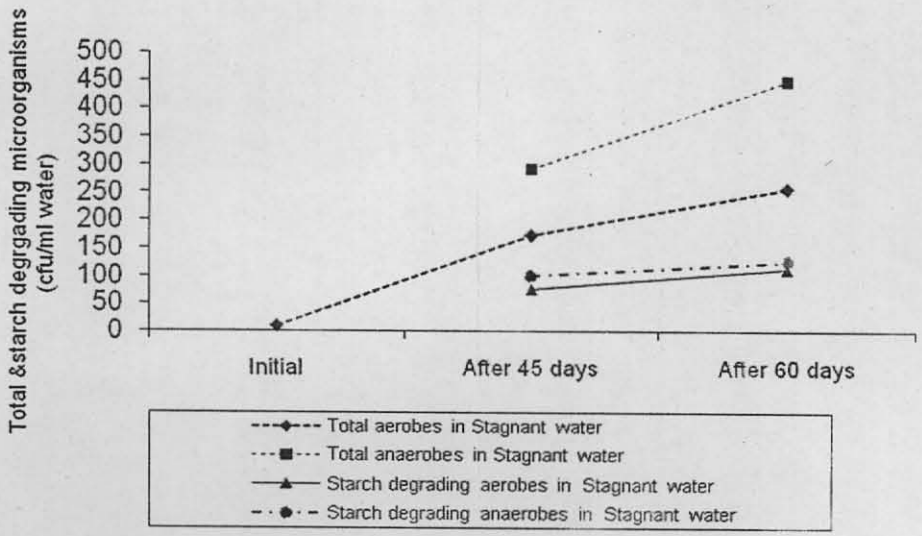

Fig. 6. Microbial population in water before and after soaking bamboo samples.

Earlier studies by Plank and Hageman (1951) and Bhat and Varma (2006) have found that the borer attack is usually restricted to starch-rich material of bamboo; bamboo culms with low starch content usually evade borer damage. Storage starch has been suggested as the key factor controlling borer damage. More intense tunneling in the inner, starch-rich portion by borer beetles and total absence of borer attack of material from bamboos that have set seeds following flowering have been cited in support of this view. Beeson (1941) has also made similar observations and has found a threshold level for starch in culms as 5 per cent; bamboo material containing starch below 5% usually escape borer damage. Results obtained from the present study have shown that the water submersion treatment is able to bring down the starch

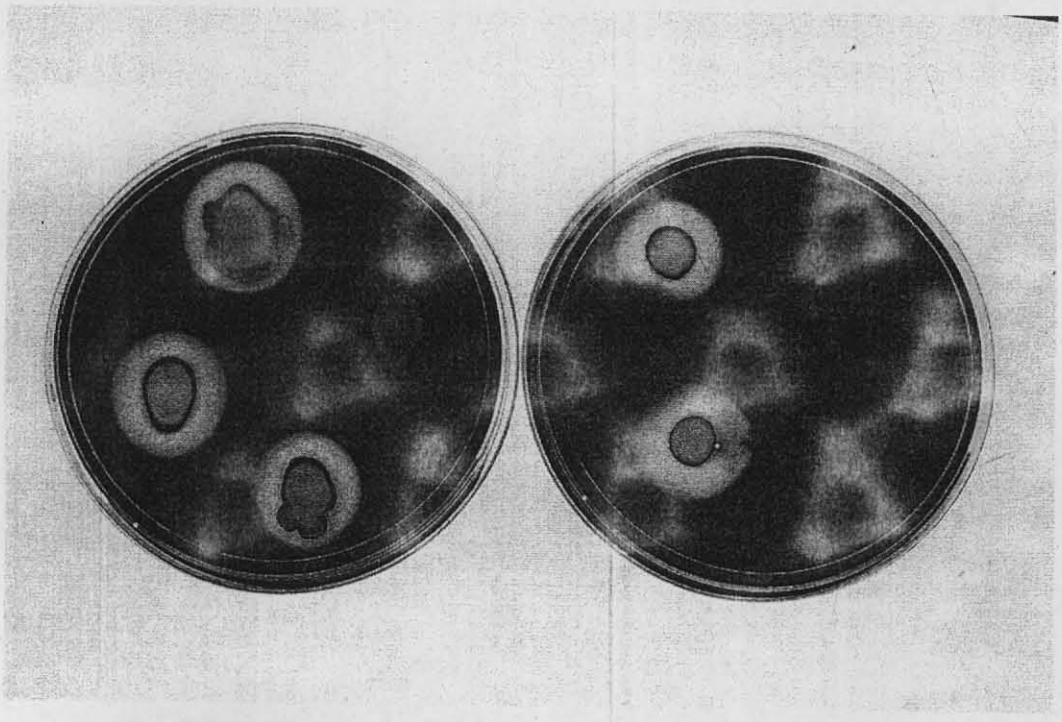

Fig. 7. Lugol's iodine-flooded starch agar plates showing the clear zone of starch hydrolysis caused by amylase enzyme secreted by the microbial colonies.

content of culms well below the suggested threshold level. Hence, it is clear that the durability imparted to bamboo by traditional water submersion treatment is due to depletion of starch brought about by the microbial population.

CONCLUSIONS

Results obtained from the present study demonstrate that the traditional method of water submersion treatment of bamboo is advantageous since it leads to starch depletion from culms. This depletion enables the bamboo to evade borer damage especially when the extent of starch in culms falls below a threshold level. The starch degradation is brought about by anaerobic and aerobic bacteria that rapidly multiply in the bamboo tissue and the water. About 75% of the bacteria were gram positive, spore forming *Bacillus* species while 25% were gram negative cocci.

REFERENCES

Beeson, C.F.C (1941). *The Ecology and Control of the Forest Insects of India and Neighbouring Countries*. Government of India, First Reprint. 767 pp.

Bhat , K.V. and Varma, R.V. (2006). *Optimization of harvesting and post-harvest technology to economize bamboo resource utilization*. KFRI Research Report 285. Kerala Forest Research Institute, Peechi 67p.

Chowdhury, M.Y. 1993. Physical and chemical treatments of bamboos for strength and durability. *In*: Zhu Shilin et al. (eds.) *Bamboo and its Use*. International Symposium on Industrial Use of Bamboo 7-11 December, 1992. China. International Tropical Timber Organisation, Yokohama and Chinese Academy of Forestry, Beijing: pp.155-157.

Hidalgo-Lopez, O. (2003). *Bamboo: The Gift of Gods*. Oscar hidalgo, Colombia

Humphreys, F.R. and Kelly, J. (1961). A method for determination of starch in wood. *Analytical Chemistry Acta* 24:66-70

Kumar, S., Shukla, K.S., Dev, T., and Dobriyal, P.B. 1994. *Bamboo preservation techniques: A Review*. Published by International Network for Bamboo and Rattan (INBAR) and Indian Council of Forestry Research Education. 26p.

Liese, W. (1980). Preservation of bamboos. In: G. Lessard, and A. Chouinard (Eds). *Bamboo Research in Asia. Proceedings of the Bamboo Workshop*. Singapore. IDRC, Ottawa, Canada. pp.161-164

Plank, H.K. (1950). Studies on the factors influencing attack and control of the bamboo powder-post beetle. *USDA For. Exp. Sta. Bull.* Puerto Rico. No. 48. 39p.

Plank, H. K. and Hageman, R. H. (1951). Starch and other carbohydrates in relation to the powder-post beetle infestation in freshly harvested bamboo. *J. Econ. Entom.* 44: 73- 75.

Sen Sarma, P.K. (1977). Insect pests and their control in rural housing. *Indian J. Entom.* 39: 284-288

Sulthoni, Achmad (1987). Traditional preservation of bamboo in Java, Indonesia, In: A.N. Rao, G. Dhanarajan and C.B. Sastry (Eds), *Recent Research on Bamboo. Proceedings 3rd International Bamboo Workshop*. Hangzhou, China, Chinese Academy of Forestry ,Beijing and IDRC, Ottawa, Canada. 349-357.

Tamolang, F.N., F.R. Lopez, J.A. Semana, R.F. Casin and Z.B. Espiloy. 1980. Properties and utilisation of Philippine bamboos. In: G. Lessard and A. Chouinard (Eds) *Bamboo Research in Asia. Proceedings of the Bamboo Workshop*. Singapore. IDRC, Ottawa, Canada. pp. 189-200.