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# Biochemical Effects Associated with Freshly Harvested Bamboo Seeds of Three Species

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**ABSTRACT**

The flowering of a bamboo is usually quite an extraordinary event, for the simple reason that it very rarely happens. They are indicators of high biodiversity, play a significant role in soil conservation and extensively used for soil and water management. Seeds of bamboos cannot be obtained every year and after seeding the bamboos die. Seeds can be used for seedling production only for short duration of maximum six months. Our aim was to observe the biochemical studies include analysis of amino acids, sugars and total phenols in all the three species of bamboos viz. *Dendrocalamus hamiltonii*, *Dendrocalamus strictus* and *Bambusa bamboos* in freshly harvested seeds. Maximum germination was found to be in case of *Dendrocalamus hamiltonii* (81.2) out of all the three species and D. strictus (76) showed minimum germination which was in correlation with the biochemical study showing analysis of amino acids, sugars, and total phenols.

## INTRODUCTION

The importance of bamboo as an eco-friendly raw material capable of meeting multifarious needs of the people at large is gaining global acceptance. From a raw material known as poor's man timber bamboo is currently being elevated to the status of “the timber of the 21$^{st}$ century". They are distinguished from another member of the family by having woody culms, complex branching and generally robust rhizome system and infrequent flowering. Bamboos are plants of global interest because of their distinctive life form, ecological importance and the range of uses and values they have for humans (Bystriakova *et al*., 2004). It is a material which accompanies one from cradle to the grave. Bamboo is the base for a broad range of rural and semi-urban cottage industries that provide the livelihood for the rural poor. At least one-third of the human race uses bamboo in one way or another. They are gaining increased attention as an alternative crop with multiple uses and benefits providing human beings living resources.They are intermingled with the tradition and culture of rural tribal populations from times immemorial due to which they have been variously called as the cradle to coffin plant' , The poor man’s Timber, Friend of the people’, Green Gasoline’, The Plant with Thousand Faces and ‘The Green Gold’, This green gold’ is sufficiently cheap and plentiful to meet the vast needs of human populace from the child’s cradle to the dead man’s grave. As a renewable natural resource, it plays a major role in the livelihood of rural people and is an integral part of our cultural, social and economic conditions (Tewari, 1988). Because of its multifarious utility, both in the traditional way for the rural people as well as in modern society, bamboo is becoming a very important plant worldwide. There are more than 1500 different document traditional uses of bamboo (INBAR, 1997). At present there are about 3,000companies around the world engaged in the production of various bamboo-based products such as panels, flooring, pulping, charcoal, edible shoots, and other daily use articles (Xuhe, 2003). Bamboos provide food, shelter, medicine, raw materials for construction, wood substitute and paper and industry.They are also used for making furniture, handicrafts, containers, tool handles, poles, musical instruments, bows and arrows, boats, rafts, fishing poles etc. The leaves have been used as fodder for livestock by the Japanese for hundreds of years. The fine-grained silica produced in the joints of bamboo stems has been used as medicine in the Orient under the name Tabasheer. The pulped fibers of several bamboo species are used to make fine quality paper.

## MATERIALS AND METHODS

Ten presoaked seeds were deglumed and weighed. Their homogenate was made in 10 ml of 80% ethanol using pestle and mortar with a pinch of acid-washed sand. It was then centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatant, thus obtained was used for the analysis of amino acids, sugars, total phenols, and ascorbic acid.

### Total free amino acids (Lee and Takahashi, 1966)

Total free amino acids were estimated by the method of lee and Takahashi (1966) using glycine as standard.To 0.5 ml of homogenate added 5 ml of ninhydrin reagent and shaken properly. The mixture was boiled for 12 minutes in the water bath and cooled thereafter. The absorbance was recorded at 570 nm on the spectrophotometer. The reference curve was prepared by using glycine (10-100µg) as std. Amino acid

### Total sugars (Yemm and Willis, 1954)

To 4 ml of chilled anthrone reagent, 0.1 ml of ethanol extract was added. Tubes were shaken gently to mix the solution. These were then covered with .glass marbles and immediately placed in a boiling water bath for 10 minutes and cooled in ice-cold water. The absorbance of the blue-green colored solution was read at 625 nm in the spectrophotometer against a blank containing 80 % ethanol extract. The concentration of standard Curve plotted with known concentrations of glucose.



### Total phenols (Bray and Thorpe, 1954)

Phenolic content was determined by the method of Bray and Thorpe (1954) with some modifications.1 gm of the seed material was extracted in 5 ml of 0.3 N HCl in methanol. Centrifuged it, retained supernatant and repeated the extraction with residue.Pooled the two supernatant and evaporated them to dryness. Dissolved the residue in 5 ml distilled water, 0.1 ml of the aliquot in each case was taken to make the final volume of 7 ml with distilled water. Added 0.5 ml of folin-phenol reagent and the solution was shaken vigorously.After 3 min.1 ml of 35%, sodium carbonate was added. The solution was shaken and then allowed to stand for 1 hour.Recorded the absorbance at 630 nm.The number of phenols was determined from an std. Curve prepared by using different concentrations of catechol and was expressed as µ/gm fresh weight

## RESULTS AND DISCUSSION

**Table 1: Levels of total Sugars (mg/gift) in seeds of three species of bamboo**

| Fresh Seeds | Seeds ageing | | |
|---|---|---|---|
| **Total Sugars** | **Time interval** | | |
| **Species** | **12 hrs** | **24 hrs** | **48 hrs** |
| ***Dendrocalamus hamiltonii*** | 2.25 | 2.34 | 2.44 |
| ***Dendrocalamus strictus*** | 2.34 | 2.45 | 2.78 |
| ***Bambusa bambos*** | 4.13 | 4.26 | 4.33 |

Total sugars were estimated by leachate analysis at three stages of soaking i.e. 12, 24 and 48 hrs at 6- monthly intervals up to 18-months of ageing. In *D.hamiltonii*, freshly harvested seeds, soaked for 12hrs and having 81.2 % germination found to be total free sugars were found to be 2.25 mg/gmfwt and in those soaked for 24 and 48 hrs 2.34 and 2.44 mg/gmmfwt respectively as seen in Table 1**.**

**Table 2: Levels of total amino acids (mg/gmfwt) in seeds of three species of bamboo**



| Fresh Seeds | Seeds ageing | | |
|---|---|---|---|
| **Total Sugars** | **Time interval** | | |
| **Species** | **12 hrs** | **24 hrs** | **48 hrs** |
| ***Dendrocalamus hamiltonii*** | 1.82 | 1.87 | 2.01 |
| ***Dendrocalamus strictus*** | 1.13 | 1.24 | 1.35 |
| ***Bambusa bambos*** | 4.24 | 4.27 | 4.33 |

Total free amino acids were estimated by leachate analysis at three stages of soaking i.e 12, 24, and 48 hrs at 6-months of intervals till 18-months of ageing. *D.hamiltonii*: In freshly harvested seeds, soaked for 1hrs, total free amino acids were found to be 1.82 mg/gmfwt at 81.2% germination and 1.87 and 2.01 mg/gmfwt those soaked for 24 and 48 hrs respectively as Table 2.

**Table 3: Levels of total phenols (mg.gmfwt) in seeds of three species of bamboos**

| Fresh Seeds | Seeds ageing | | |
|---|---|---|---|
| Total Sugars | Time interval | | |
| Species | 12Hrs | 24 Hrs | 48 Hrs |
| ***Dendrocalamus hamiltonii*** | 0.49 | 0.53 | 0.58 |
| ***Dendrocalamus strictus*** | 0.23 | 0.24 | 0.27 |
| ***Bambusa bambos*** | 0.20 | 0.22 | 0.26 |

Total phenols were estimated by leachate analysis at three stages of soaking i.e 12, 24 and 48 hrs at 6-months of intervals until 18-months of ageing. *D. hamiltonii***:** In freshly harvested seeds soaked for 12 hrs. Total phenols were found to be 0.49 mg/gmfwt and 0.53, 0.58 after 24 and 48 hrs of imbibition respectively as in Table 3. Seed ageing is a time- depended process in which the seeds deteriorate during periods of prolonged storage and eventually lose their ability to germinate. Seed ageing is a natural phenomenon which occurs in all seeds, even if they are stored in dry and low-temperature rooms( Machado Neto *et al*., 2001). The main factors affecting seed are storage, the moisture content of the seeds and the seed quality. It is a process that can occur slowly in dry systems and might be expected to contribute to dynamic ageing especially in accelerated ageing condition (Narayana Murty *et al*., 2000). In dry seeds, the protein modification can take place through non-enzymatic glycation with reducing sugar ( Sun and Lopold,1995) and by reacting with α, β- unsaturated aldehydes which are products of the free radical-mediated oxidation of polyunsaturated fatty acids (Priestley 1986)and also α, β unsaturated aldehydes , especially 4-hydroxynonenal may react with the sulfhydryl I group of proteins to form stable thioether derivatives that sulphydryl group of proteins to form stable thioether derivatives that possess a carbonyl function (Stadtman,1992). It was reported that wheat seed when stored at 35°C and 12.5-14.5% methionine uptake and incorporation into soluble proteins of the imbibing embryos. Lower number of soluble proteins were present in the non- viable seed than in viable ones. This showed that the proteins with higher mobility were denatured during the ageing process.( Chiu *et al.*,1995) reported that watermelon seeds when incubated at 45°C and 79% Rh for 6- days, showed lower germination percentage and slower germination speed. Ageing also increased lipid peroxidation and reduced the activity of peroxide-scavenging enzymes. Abdul- Baki (1980) reported that a decrease in seed germination and vigour induced by ageing were associated with marked decrease in synthesis of protein and polysaccharides.

Bucharov and Gantcheff (1984) reported a decrease in total protein content with concomitant increase in amino acid in accelerated aged seeds of *Phaseolus vulgaris, Pisum sativum, Lens culinaris and Panicum miliaceum.* An increase in soluble carbohydrates in seed of rice, nonreducing sugar (Walters *et al*.2008) and glucose in wheat seeds (Petruzzelli, 1986) was reported during ageing.

## CONCLUSION

Biochemical studies included changes in the levels of various metabolites in leachates such as free sugars, amino acids, phenols. Biochemical analysis showed increase in the levels of total sugars, free amino acids, and phenols in leachates. This could be contributed to a greater degradation of reserve food materials in free utilizable forms. The significant proportional correlation could be drawn between reserve mobilization and seeds ageing which may be due to the membrane deterioration which in turn leads to excessive leakage of various metabolites during imbibitions with ageing**.**

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