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Effects of Culm height and growth on the Cyanogenic Glycosides content of *Thyrsostachys oliveri* Gamble

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Thyrsostachys oliveri shoots, growth, cyanogenic glycosides

A B S T R A C T

The emerging fresh young delicate bamboo shoots of *Thyrsostachys oliveri* Gamble are used as delicious and nutritive food. However bamboo contain cyanogenic glycoside (HCN) which are toxic to consumers. Therefore evaluation of cyanogenic level in the plant is necessary before its consumption as food. In order to study the cyanogenic activity in the young stages of plants and at maturity, the culms growth length and phenology of the bamboo species *Thyrsostachys oliveri* and the cyanogenic content during its growth were studied for two consecutive years 2010 and 2011. in which it was observed that the growth rate of the bamboo *Thyrsostachys oliveri* was slow when the culms just emerged from the ground (up to the first ten days) in which the shoot is tender and completely covered by culms sheath with no internodes seen outside. However with increase in days the growth rate increases with increase in height up to a certain period and then declines showing a sigmoidal curve in its growth rate. The cyanogenic glycosides content was minimum in emerging young fresh succulent shoots and increased with increase in age of the shoot harvested up to four weeks and declines thereafter. Also the cyanide content were assessed during fermentation processing of these bamboo shoot slices since bamboo shoots were consumed both in fresh and fermented form . In the fermentation of the bamboo shoot slices, it also shows a degradation of HCN content with the advance of fermentation as compared to the fresh unfermented bamboo shoot slices.

Introduction

Bamboo is a group of woody perennial evergreen plants in the grass family Poaceae, subfamily Bambusoideae, and tribe Bambuseae and are specially remarkable by containing those large tree like members of the family belonging to order monocotyledon (Abd latif *et al*., 1990). Bamboos are well known for their fast growth rate having a growth rate of 4.7 inches/day (Farrelly, 1984) but they shows great variation in their heights and diameter of the culm (Naithani, 2008). Bamboo shoots are the young culms emerging from nodes of the rhizome of bamboo plant. The fresh young bamboo shoots are harvested just as it appears above the ground and used as vegetables. They are used in numerous Asian dishes and are available in markets in various sliced forms, fresh, fermented and canned version (Tai, 1985; Midmore, 1998). The utilization of bamboo shoot for fermentation, roasted and boiled shoots was estimated to be approximately 680 tones in the north eastern state (Bhatt *et al.*, 2003). In Manipur, young emerging fresh bamboo shoots are treated as one of the delicious food item. More than 700,000 culms are extracted every year in Manipur (Statistical bulletin of Manipur forest, Govt. of Manipur, 1999-2000). Bamboo shoots are consumed in different form as fresh, fermented, pickled, dried or canned. But the bamboo shoots are found to contain cyanogenic glycosides releasing hydrogen cyanide (HCN), a well-known toxic compound, which is released from cyanogenic glycosides which upon consumption via acid and enzymatic hydrolysis encountered during digestion. The high amount of cyanogens in some plants, such as cassava and flaxseed meal, may causes health concerns and limits the utilization of these plants and their products for human and animal consumption (Wanasundara & Shahidi 1998; O'Brien *et al.,* 1992). Major cyanogenic compounds associated with food plants are amygdalin, dhurrin, linamarin, linustatin, prunasin and taxiphyllin (Conn, 1969; Nielsen *et al*., 2011; Haque and Howard Bradbury, 2002; Simeonova and Fishbein, 2004). The cyanogenic glycoside in bamboo is taxiphyllin which is a p-hydroxylate mandelo-nitrile triglochinin (Nahrstedt, 1993; Schwarzmaier, 1997; Pandey and Ojha, 2013) and therefore one of the few of these cyanogenic compounds that decomposes quickly when placed in boiling water. Bamboo becomes edible because of these properties (Nahrstedt, 1993). Taxiphyllin is a bitter compound (Ke-jun et al., 2005) making some bamboo shoots taste bitter to eat. It has also been reported that the level of cyanogenic glycosides produced is dependent upon the age, variety and parts of the plant, as well as environmental factors (Cooper-Driver and Swain, 1976; Woodhead and Bernays, 1977). The present investigation is undertaken to assess the concentration of cyanogenic glycosides content in the culms/bamboo shoots at different harvesting ages during growth period of the bamboo plant (*Thyrsostachys oliveri*). Also the cyanide content were assessed during fermentation processing of these bamboo shoot slices since bamboo shoots were consumed both in fresh and fermented form.

Materials and Methods

The study was conducted in the village of N. Molhoi Churachandpur district, Manipur having latitude 23.0-56′20.4 north and longitude 24.0-36′46.8 east. The average annual rainfall is 3050 mm, most of which is received during the month of May to July. The species *Thyrsostachys oliveri* Gamble was selected randomly. New shoots or culms sprouting one each from the clumps were taken for daily growth analysis and other phenology such as culms sheath appearing, culms sheath falling, diameter of the culms base which was measured after sheath uncover the culms. Branching and leafing characteristics was studied for 3 months consecutively for two years, 2010 and 2011. New shoots or culms sprouting one each from the clumps were taken for daily growth analysis.

For cyanide estimation, the newly emerging shoots of *Thyrsostachys oliveri* at different ages (7 days onward till 90 days), were collected from the natural habitat during morning hour as transpiration is less. Length of fresh shoots before harvesting were recorded and after removing sheaths the edible fresh delicate portion was taken and analyzed for their hydrogen cyanide content so as to assess the content of cyanogen varying with different age and to determine the suitable ages for harvesting of the bamboo shoots for consumption.

Analysis for cyanogenic glycoside

Cyanogenic glycosides estimation was done using the technique of Picrate-impregnated paper according to Bradbury *et al*., 1999. The assay was performed in triplicate. Fresh bamboo shoots was cut into small pieces and crushed in a pestle and mortal and immediately placed into a small flat bottomed vial. 0.5ml of phosphate buffer (0.1M, pH 7) and 5 drops of chloroform was added followed by brief stirring with a glass rod. A picrate paper was added and the vial immediately closed with a screw stopper and left for 18-20 hour at room temperature.

The liberation of HCN is indicated by the colour change of picrate paper from yellow to reddish or red brown colour in proportion to the amount of HCN released. The picrate paper was then removed and the colour eluted in a clean test tube containing 5ml of distilled water and kept at room temperature for 30min. The absorbance was measured at 510nm and the total cyanide content was determined by preparing standard curved from potassium cyanide.

Picrate paper preparation: Strips of filter paper (12×1cm) were soaked in an aqueous solution of 0.05M picric acid, previously neutralized with sodium bicarbonate and filtered. The impregnated paper was left to dry at ambient temperature.

Fermentation

Fermentation of the fresh bamboo shoot slices were carried out in the laboratory by a modified form of the traditional method of fermentation which involves inoculating thin slices of the succulent bamboo shoots with the exudates obtained from already fermented slices of bamboo shoots (traditionally fermented) under aseptic condition using a Laminar flow. After inoculation, the samples were kept in an incubator at 30 ± 2^{o}C for a period of 60 days. During this period of fermentation, interval analysis on cyanogenic content was conducted.

Statistical analysis

All data were statistically evaluated with SPSS (Version 14.0) software. Data are expressed as means ± SD. Various treatment groups were subjected to one way analysis of variance (ANOVA) test followed by Tukey's significant difference post hoc comparison procedures. A probability of less than 0.05 ($p < 0.05$) was considered significant in the present study.

Results and Discussion

The shoots of the bamboo plant *Thyrsostachys oliveri* takes 5-7 days to emerge above the ground after breaking the earth. It is also observed that growth is slow during this period up to the first ten days the shoot is tender which is completely covered by culms sheath with no internodes seen outside (Fig.1a and fig.2b). However the next 45 days, the growth rate increases in spurts of increasing height of 8cm to 27cm per day. Again from the next 45 days growth rate decreases to 7cm –5cm a day. Almost the total growth height is attained during these forty-five days (fig.2) and when plotted on graph it shows sigmoid curve (Fig3).

It was also found that the number of culms sheath increases with increases in length (4-25 sheath). At the initial stage the shoot is covered by 4 sheaths, then as the height increases the number of culms sheath reach up to 24 sheaths. The diameter of the culms was measured when the base culms was uncovered by sheath or when the culms was naked because of up growth of the sheath, and it was found that culms diameter at the base was ranging from 14cm to 23.30cm and node having 2 to 23 also increases with increase in height. Branching and leafing was observed towards the ends of ninety days. Similar trends was also reported with that of *Bambusa bamboos* where branch and leaf appear on the sixty to ninety day and the culms may become fully leafy in about one hundred fifty to one hundred eighty days (Shanmughavel and Francis, 2001). Unlike other bamboos, no culms sheath falling was recorded in *Thyrsostachys oliveri* within these 3 months of study periods.

Changes in the HCN content with aging of the succulent bamboo shoots in their natural habitat and growth of the bamboo were also studied. The cyanogen content was minimum in emerging fresh succulent shoots and increased with increase in age of the shoot harvested and the concentration of the HCN decline after four week (Table 1). In the study on the hydrogen cyanide content at different harvesting ages of the bamboo shoots of *Thyrsostachys oliveri* there is significant difference between weeks as well as between plant parts.

Table.1 Changes in the cyanogenic glycosides (HCN) content in the bamboo shoots of *Thyrsostachys oliveri* at different harvesting ages during growth in the natural habitat

Bamboo shoots harvesting period (weeks old)	Portion of the soft edible bamboo shoots	cyanogenic glycosides (HCN) mg/100g fresh wt.
1	Apex	34.67±2.31
	Base	7.33±1.53
	Middle	16.00±1.73
2	Apex	31.33±1.53
	Base	17.33±0.58
	Middle	22.00±1.73
3	Apex	44.00±1.00
	Base	16.33±1.53
	Middle	28.00±1.00
4	Apex	49.67±1.15
	Base	23.33±2.52
	Middle	72.33±1.53
5	Apex	26.33±1.15
	Base	32.67±3.06
	Middle	56.00±1.73
6	Apex	18.00±1.73
	Base	23.00±3.61
	Middle	33.33±2.52

* Data presented as mean ± SD.

Fig:1(a)

Fig:1(b)

Fig.1 a)Measuring the growth length of the bamboo shoots
b)Fully emerged fresh bamboo shoots of *Thyrsostachys oliveri*

Fig.2 Showing the measurement of bamboo growth of Thyrsostachys oliveri. Gamble

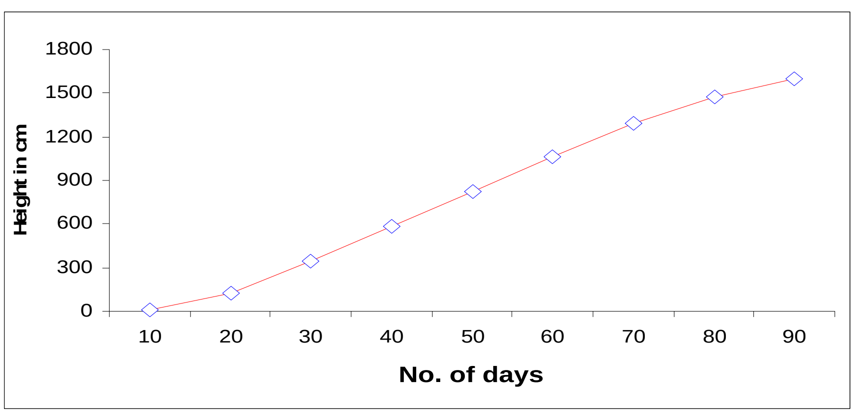

Fig.3 Showing the growth in height (cm) observed during 3 months on the growth of the culm/shoot of *Thyrsostachys oliveri*

Table.2 Multiple Comparisons of cyanogenic glycosides (HCN) content in the bamboo shoots of *Thyrsostachys oliveri* at different harvesting ages during growth in the natural habitat.
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-4.2222*	.90722	.001	-6.9517	-1.4928
	3	-10.1111*	.90722	.000	-12.8405	-7.3817
	4	-29.1111*	.90722	.000	-31.8405	-26.3817
	5	-19.0000*	.90722	.000	-21.7294	-16.2706
	6	-5.4444*	.90722	.000	-8.1739	-2.7150
2	1	4.2222*	.90722	.001	1.4928	6.9517
	3	-5.8889*	.90722	.000	-8.6183	-3.1595
	4	-24.8889*	.90722	.000	-27.6183	-22.1595
	5	-14.7778*	.90722	.000	-17.5072	-12.0483
	6	-1.2222	.90722	.757	-3.9517	1.5072
3	1	10.1111*	.90722	.000	7.3817	12.8405
	2	5.8889*	.90722	.000	3.1595	8.6183
	4	-19.0000*	.90722	.000	-21.7294	-16.2706
	5	-8.8889*	.90722	.000	-11.6183	-6.1595
	6	4.6667*	.90722	.000	1.9372	7.3961
4	1	29.1111*	.90722	.000	26.3817	31.8405
	2	24.8889*	.90722	.000	22.1595	27.6183
	3	19.0000*	.90722	.000	16.2706	21.7294
	5	10.1111*	.90722	.000	7.3817	12.8405
	6	23.6667*	.90722	.000	20.9372	26.3961
5	1	19.0000*	.90722	.000	16.2706	21.7294
	2	14.7778*	.90722	.000	12.0483	17.5072
	3	8.8889*	.90722	.000	6.1595	11.6183
	4	-10.1111*	.90722	.000	-12.8405	-7.3817
	6	13.5556*	.90722	.000	10.8261	16.2850
6	1	5.4444*	.90722	.000	2.7150	8.1739
	2	1.2222	.90722	.757	-1.5072	3.9517
	3	-4.6667*	.90722	.000	-7.3961	-1.9372
	4	-23.6667*	.90722	.000	-26.3961	-20.9372
	5	-13.5556*	.90722	.000	-16.2850	-10.8261

Based on observed means
The error term is Mean Square (Error) = 3.704.
*The mean difference is significant at the .05 level.

Table.3 *Tukey HSD* between different plant parts *Thyrsostachys oliveri* during growth

(I) Plant Part	(J) Plant Part	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Apex	Base	14.0000*	.64150	.000	12.4320	15.5680
	Middle	-3.9444*	.64150	.000	-5.5125	-2.3764
Base	Apex	-14.0000*	.64150	.000	-15.5680	-12.4320
	Middle	-17.9444*	.64150	.000	-19.5125	-16.3764
Middle	Apex	3.9444*	.64150	.000	2.3764	5.5125
	Base	17.9444*	.64150	.000	16.3764	19.5125

Based on observed means.
The error term is Mean Square (Error) = 3.704.
*The mean difference is significant at the 0.05 level.

Table.4 Changes in the cyanogenic glycosides (HCN) content during fermentation of the bamboo shoot slices of *Thyrsostachys oliveri*

Fermentation period (Day/s)	*Thyrsostachys oliveri* mg/100g fresh wt.
0	34.12±1.08
7	32.10±1.00
14	23.00±1.65
21	16.00±1.00
28	14.00±1.08
35	11.00±1.65
42	08.76±1.73
49	09.00±1.00
56	09.00±1.65
60	09.00±1.58

*Data presented as mean ± SD.

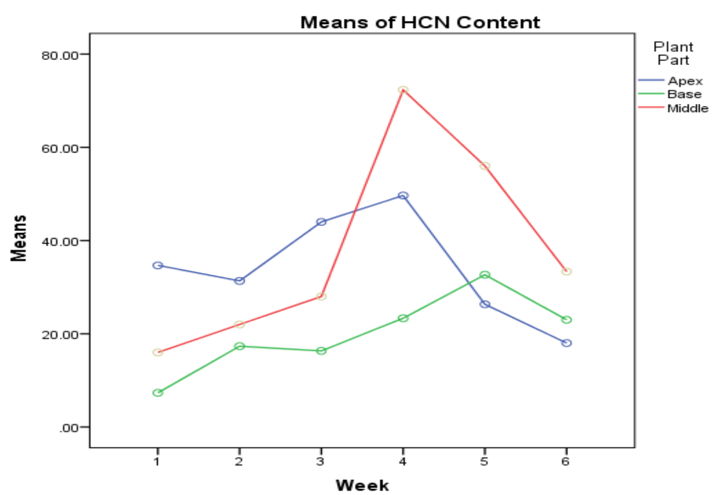

Fig.4 Profile Plots of the cyanogenic glycosides (HCN) content in the bamboo shoots of *Thyrsostachys oliveri* at different harvesting ages during growth in the natural habitat

The apex portion of the soft edible bamboo shoots which are bitter in taste have high cyanogenic glycosides(HCN) whereas the base portion of the bamboo shoots were found to have lesser amount of HCN as is shown in Table 1. In the multiple comparisons (Table 2) there is no significant difference between HCN content in 2nd week and 6th week. In other weeks, there are significant differences. HCN content falls abruptly in 6th week to the level of 2nd week. And according to the

Tukey HSD (Table 3) between different plant parts, there are significant differences. A profile plot of the hydrogen cyanide content in *Thyrsostachys oliveri* at different harvesting ages during growth in the natural habitat is shown in Fig.4. The present finding shows relevant to the previous report that the level of cyanogenic glycosides produced is dependent upon the parts of the plant and age (Cooper-Driver and Swain 1976; Woodhead and Bernays, 1977).

Changes in the cyanogenic content during fermentation of the bamboo shoot slices of *Thyrsostachys oliveri* in the modified laboratory fermentation were conducted. The weekly analysis on the hydrogen cyanide content assessed for 60 days with the bamboo shoot slices of *Thyrsostachys oliveri* shows a decreasing trend of hydrogen cyanide level from34.12mg/100g to 09 mg/100g fresh wt. In all days of fermentation it shows a degradation of HCN content with the advance of fermentation. Fermentation may be the process for detoxification of the toxic component of cyanogenic glycosides

Conclusion

In the study on the hydrogen cyanide content at different harvesting ages of the bamboo shoots of *Thyrsostachys oliveri* there is significant difference between weeks as well as between plant parts .The cyanogen content was minimum in emerging fresh succulent shoots and increased with increase in age of the shoot harvested and the concentration of the HCN decline after four week. HCN content falls abruptly in 6th week hence harvesting of the edible shoot should be appropriate during age of 4-6 weeks old shoots. The fermenting process of bamboo shoot slices reduce significantly the amount of cyanogenic glycosides content. Hence fermentation technology both in traditional and scientific methods should be encouraged to reduce the consumption of toxic components present in fresh bamboo shoots.

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