



## ESTIMATION OF BIOCHEMICAL CHANGES IN SUGARCANE DUE TO POKKAH BOENG DISEASE

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### Abstract

Sugarcane is an important cash crop and has been grown in India since the ancient time. It is mainly utilized to prepare sugar. Sugarcane production shows reduction due to many biotic stresses in the form of disease caused by various microbes. Although pokkah boeng is a minor disease caused by *Fusarium moniliforme* but recently the incidence of pokkah boeng disease have been increased and most of the sugarcane varieties got affected ranged from 1-90%. The present study aimed to observe the changes in amino-acid and protein content of sugarcane due to pokkah boeng disease. Five sugarcane varieties were taken viz. CoS 8436, CoS 8432, CoS 98259, CoLk 8102 and CoSe 01434. Data revealed that amino acid content was significantly higher in all test varieties of sugarcane in comparison to that of healthy canes. But ascorbic acid and protein content in all test varieties of sugarcane was significantly higher in healthy canes in comparison to that of diseased canes.

**Keywords:** *Fusarium moniliforme*, Pokkah boeng, Sugarcane, Biochemical Estimation.

### Introduction

Sugarcane has an old association with India. It has been grown in India since the ancient time and has its reference in the Vedic literature (500 B.C) (Sato, 2014). Chinese writers of the 8<sup>th</sup> century have recorded that knowledge of sugarcane and its products were derived from India (Sidney, 1986). Sugarcane is also known as *Ganna* or *Ekh* in vernacular language. In general, *S. officinarum* is known as good cane whereas *S. robustum* as a wild cane. It is believed that India is the original home of sugarcane (Daniel and Daniel, 1993; Paterson *et al.* 2012; Kumar and Dwivedi, 2018a; Kumar *et al.*, 2018b; Kumar *et al.*, 2018c; Kumar and Dwivedi, 2018d; Kumar and Purnima *et al.*, 2018e; Kumar and Pathak, 2019f; Kumar *et al.*, 2019g; Siddique and Kumar, 2018h; Siddique *et al.*, 2018i; Pathak *et al.*, 2017j; Prakash and Kumar, 2017k; Kumar and Mandal, 2014L) and the indigenous Indian cane species is botanically known as *Saccharum barberi* Jews. These canes are thin or medium in thickness. The thicker class of canes is botanically known as *Saccharum officinarum* L. and known initially to grow in Mauritius, Java, Brazil and West Indies from South Pacific origin (Fauconnier, 1993). Sugarcane plays a vital role in Indian economy by providing direct and indirect employment, and due to its perennial nature and dependable remunerative crop.

Globally, about 240 diseases have been recorded on sugarcane (Rott *et al.*, 2000). However, amongst them, the major diseases are 40 fungal, 5 bacterial, 8 viral, 3 nematodes, and 8 miscellaneous diseases as per American Phytopathological Society Report. Primarily, sugarcane diseases are grouped into two categories namely, "Seed transmitted" and "Non seed transmitted". The former group includes all viral, mycoplasmal, fungal and bacterial diseases which are responsible for heavy yield losses, varietal decline and deterioration of the seed stocks in India (Rao *et al.* 2002). The second group includes minor diseases like, leaf spots, blights, rust and root- rot etc. which are neither severe nor of widely occurring and have only seasonal importance in certain areas. About 10–15% of the nation sugar produced is lost due to the diseases (Viswanathan and Rao, 2011;

Kumar *et al.*, 2014m; Kumar *et al.*, 2014n; Kumar, 2013o; Kumar and Dwivedi, 2015p; Gogia *et al.*, 2014q; Kumar, 2014r; Kumar *et al.*, 2012s; Mishra *et al.*, 2012t; Kumar *et al.*, 2011u; Kumar *et al.*, 2011v; Kumar *et al.*, 2016x). The economic value and distribution of all diseases occurred on the sugarcane varieties and the agro-climatic conditions under which they are grown, are defined by Alexander and Vishanathan (2011).

Yield of sugarcane is hindered by diseases mostly as a result of its vegetative propagation through setts, resulting in easy dissemination of causal organism (Pierre *et al.* 2014). Out of all diseases of sugarcane, although Pokkah boeng comes under insignificant concern but now a days it is considered as a major disease on basis of their frequent spread since last few years (Vishwakarma *et al.* 2013). The identified causal organism of the mentioned disease is *Fusarium moniliforme* (Lin *et al.* 2014; Zhang and Jeyakumar, 2018). Following resistant cultivars of sugarcane have been recommended by the scientists, these are Co 0238, Co 0118, CoS 08279, CoS 8436, CoS 08272, CoS 96268, CoSe 03234, CoSe 98231, CoSe 01235 and CoSe 92423. In addition, mid-late cultivars namely CoS 96275, CoS 97261, CoSe 95422, UP 05125, CoS 98259, CoSe 01424, CoS 91230, CoS 92263, CoS 94257, and two varieties viz., UP 9530, CoSe 96436 have also been recommended for waterlogged areas. This article aimed to observe the changes in amino-acid and protein content of sugarcane due to pokkah boeng disease.

### Materials and Methods

To understand the nutritional value, like biochemical contents in both healthy and diseased sugarcanes, various biochemical experiments were carried out in the laboratory after collecting the samples from the experimental field. Healthy and infected sugarcane samples were collected from experimental field in sterilized polythene bags and brought to laboratory for study. The equipments used in study were Conical flask, Test tube, Hemocytometer, Sugar tubes, Micropipettes, GDW, Hot plate, Spectrophotometer, Dextrose, Liquid Nitrogen and Mortar pestle etc.

### Amino acid content in healthy and infected cane varieties

The same general method conducted for estimation of soluble sugar as described was followed. 10 ml of each of the test sample solution was obtained. The amount of amino acid present was determined by the modified method adopted by Maehre *et al.* (2016).

The optical density was taken at 570 nm absorbance by using UV-VIS spectrophotometer against blank. The total amino acid content was evaluated by making comparison with the standard curve of a known amino acid.

The result data on estimation of amino acid contents in healthy and diseased test varieties of sugarcane is given Table-1. Data revealed that amino acid content was significantly higher in all diseased samples of test varieties of sugarcane in comparison to that of healthy canes.

**Table 1:** Comparison of Amino acid between healthy and infected cane

S. No.	Varieties	Amino acid (ug/100mg fwt.)	
		Healthy	Disease
1.	CoS 8436	220.763	285.766
2.	CoS 8432	225.406	300.426
3.	CoS 98259	126.246	288.933
4.	CoLk 8102	263.842	277.433
5.	CoSe 01434	226.653	285.51

### Ascorbic acid content in healthy and infected cane varieties

1gm of each sample was taken separately for the estimation of ascorbic acid content. The samples were crushed with 6% Metaphosphoric acid. The homogenate was transferred to centrifuge tubes and centrifuged at 5000 rpm for 10 minutes. The supernatants were made up to the volume of 5ml and these were used for the spectrophotometric estimation.

The optical density was taken at 660 nm absorbance in UV-VIS spectrophotometer against blank. The ascorbic acid content was estimated with the help of standard curve of known ascorbic acid. The result data on estimation of ascorbic acid content in healthy and diseased cane samples of all test varieties of sugarcane is given in Table-2.

**Total 2:** Comparison of Ascorbic acid between healthy and infected cane

S. No.	Varieties	Ascorbic acid (ug/100mg fwt.)	
		Healthy	Disease
1.	CoS 8436	16.073	36.54
2.	CoS 8432	18.163	34.233
3.	CoS 98259	21.661	35.88
4.	CoLk 8102	14.653	37.186
5.	CoSe 01434	16.976	34.055

Result data revealed that ascorbic acid content in diseased samples of all test varieties of sugarcane was significantly higher in healthy canes in comparison to that of diseased canes. This trend showed that there was emergence of higher concentration of ascorbic acid with the progression of disease development (Kumar *et al.*, 2018y; Kumar *et al.*, 2018z; Kumar *et al.*, 2018aa; Kumar *et al.* 2018bb; Kumar *et al.*, 2018cc; Singh *et al.*, 2020a; Singh *et al.*, 2020b; Sood *et al.*, 2020; Bhadrecha *et al.*, 2020; Singh *et al.*, 2020c; Sharma

*et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019; Sharma *et al.*, 2019).

### Protein content in healthy and infected cane varieties

The samples weighing 1 gm each was taken out separately then 5ml of 20% TCA was added and crushed using mortar and pestle. The crushed samples were transferred for centrifugation at 5000 rpm for 15 minutes. The supernatant was discarded and 3ml of ethanol was added to the residue part. Using a glass rod the residues were mixed properly and kept for 10 minutes at the room temperature. Then the tubes were again centrifuged and discard the supernatant. Add 2ml of 0.3N NaOH in the residual parts and kept in the incubator at below 40°C for 24 hours before the estimation of protein content (Das *et al.*, 2010).

Absorbency was taken at 750nm in 200–20 UV-VIS, spectrophotometer against a blank. The protein content was calculated by comparing with the standard curve of a known protein (*i.e.* Bovine serum albumin)

The result data on protein contents in healthy as well as diseased cane samples of all test varieties of sugarcane is given in Table-3. The data revealed that protein content in healthy cane was significantly higher than that of diseased cane samples.

**Table 3:** Comparison of Protein between healthy and infected cane

S. No.	Varieties	Protein (ug/100mg fwt.)	
		Healthy	Disease
1.	CoS 8436	2.476	1.579
2.	CoS 8432	2.846	1.746
3.	CoS 98259	2.481	1.793
4.	CoLk 8102	2.406	1.243
5.	CoSe 01434	2.862	1.264

Sugarcane juice contains significantly higher concentrations of sugars than the proteins. Therefore, the analysis of proteins by UV-visible spectrometry results in inaccurate estimation due to the interference of sugars and amino acids ion exchange for chromatography and protein pontification are changed for enzyme activity in condition of disease development of healthy and pokkah boeng infected each cane variety.

The amount of amino acids present was determined by the modified method (Bhatia *et al.*, 2012). In the present study result revealed that amino acid content was significantly higher in the pokkah boeng infected sugarcane in comparison to that of healthy canes. Microbes instantly consumed free amino acids in contrary to secreting protease enzyme utilized for the degradation of protein into smaller units, *i.e.*, amino acids. With the insufficiency of free amino acids, they perform an important role in the consumption of protein (Das *et al.*, 2010). The degradation process in nitrogenous compounds was not as prolific as was determined in the case of carbohydrates (Rodriguez *et al.* 1999; Esti *et al.*, 2002).

Estimation of ascorbic acid content is significantly higher in healthy cane with comparison to diseased canes. This trend showed that there was emergence of higher concentration of ascorbic acid with the progression of disease development. The supernatants were made up to the volume of 5ml and these were used for the spectrophotometric

estimation (Bhatia *et al.*, 2012). Das *et al.* (2010) who has described the ascorbic acid or vitamin C content got decreased during biotic stress because it is considered as a potential scavenger of free radicals, so subsequently used by microbes for their own defence system under high moisture content. It was also observed by many scientists that ascorbic-acid content also decreased during long-term (Howard *et al.*, 1999; Lee and Kader, 2000). These are considered as important for the biological and structural organization of each and every living organism (Rao, 2003).

In the present study, the result revealed that protein content in healthy cane was significantly higher concentrations of sugars than proteins. Therefore, the analysis of proteins by UV-visible spectrometry results in inaccurate estimation due to the interference of sugars and amino acids ion exchange for chromatography and protein pontificating are change for enzyme activity in condition of disease development of healthy and pokkah boeng infected of *F. moniliforme*. Das *et al.* (2010). They have represented that protein degradation was somewhat slower during primal period of time but later on it became faster, role in protein consumption. The lipid content was broken down into fatty acids or glycerol and utilized (Mowlah and Itoo, 1983).

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