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Comparative analysis of Sugars in *Mangifera indica* L. relative to quick decline disease

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Abstract
Mango sudden death syndrome (MSDS) commonly known as Quick Decline Disease has been a threat to Mango production in the world. The present phytochemical study aimed to investigate the sugars in bark of healthy, adjacent to disease and diseased *Mangifera indica* (Mango) variety Langra. Ion exchange chromatographic technique showed presence of xylose, fructose, arabinose, glucose, lactose and an unidentified sugar in barks' extracts. The non-uniform distribution of these sugars was supported by the quantitative analysis of sugar content in the samples. The results revealed the difference in total sugar content in healthy, adjacent to disease and diseased samples. Reducing sugars showed an interesting decrease in adjacent to disease samples while having a slight elevation in the diseased plant's samples. The non-reducing sugars showed a steady decrease from healthy to diseased plant's samples. Consequently, the present research showed sugars as markers in Mango Plant variety Langra in relation to Quick Decline.

Keywords: Noni fruits, Extraction, total antioxidant activity, total phenols

1. Introduction
Mango (*Mangifera indica L.* of Family Anacardiaceae) is one the most famous fruits in the world. It's known for its delicious taste and is often named as King of fruits [1]. Tropical and subtropical climates are favorable for the production of Mangoes [2]. Due to favorable location and climate, Pakistan is the 6th biggest mango-producing country [3] and 5th biggest mango-exporting country [4] in the world. Mangoes are reflected as great dietary source [5], rich in carbohydrates, amino acids, fatty acids and Vitamins. They are also well-known for dietary antioxidants [6]. According to the recent studies, Mango plant offers more than refreshingly delicious fruits. Its various pharmacological and industrial applications have been noted. Various phytochemicals from *Mangifera indica L.* have shown pharmacological properties such as antiallergic and anthelmintic [7], therapeutic [8], genotoxic [9] and antimicrobial properties [10, 11] and cytoprotective effects [12].
In last decade, the mangoes' yield in Pakistan has decreased despite of her suitable climatic and soil conditions for production of mangoes. Total 27 diseases [5] threaten the Pakistan's mango industry, among which Mango sudden death syndrome (MSDS) commonly known as Quick Decline has been dominant [13]. It is also technically known as "Collar or Stem rot" [5]. Quick Decline can destroy a healthy mango plant within a few days [2]. The first pathogenic fungus associated with quick decline in Pakistan was found to be *Ceratocystis fimbriata* [14]. Same species were identified in Brazil [15] and Oman [16]. In a detailed survey, *Lasiodiplodia theobromae* (*Pat.*) [17], *Ceratocystis fimbriata*, and *Phomopsis sp.* [14] were recognized as main destructive plant pathogens causing Quick Decline in Mangoes in Pakistan [13]. Most prominent symptoms associated with Quick Decline in mangoes include: bark's gummosis, splitting of bark, vascular discoloration [18], blackening and blotching of small shoots and vascular drying [19].
Considering the current challenges faced by mango industry in the Pakistan and the World, the present study aimed to address the changes in Sugar content in bark of Mango variety Langra, before and after the occurrence of disease. The qualitative and quantitative estimations were compared between the healthy, adjacent to disease and diseased barks of Mango variety Langra. Such information would be helpful to understand the effects of Quick decline on the mango plant and its defense mechanism against it. It may also be useful to construct a strategic approach to produce remedy for Quick decline disease and thus prevent the mango industry from loss.

2. Materials and Methods

Sampling: The bark samples of healthy, adjacent to disease and diseased plants of mango variety Langra were collected from the Experimental orchids of Mango Research Station Shujabad, Punjab, Pakistan. The samples were shade-dried and grounded with electrical grinder to powder. Samples were labeled as Healthy bark (S_a, S_b and S_c), Adjacent to diseased bark (S_d, S_e, and S_f) and diseased (S_g, S_h and S_i) and were stored in plastic bottles for study.

Extraction of sugar and amino acids: One gram of each grounded sample was soaked separately in 40 ml of 75% alcohol for 24 hours. The soaked samples were further grounded for 2 hours and then filtered. The residue was rinsed with few ml of 75% alcohol and the filtrate's volume was made up to 100ml. The filtrate of each sample was tested for sugar contents with Molisch reagent [20]. The test was positive for each sample. Then the filtrates were preserved in refrigerator for separation of sugars from free amino acids to specifically analyze the sugar contents in the samples.

Separation of Sugars from free amino acids: Ion exchange chromatographic technique was used to separate amino acid and sugar contents from the extracts obtained from each sample. The following method was implied to all 9 extracts. A 73 cm long column with the diameter of 4cm was packed with regenerated resin of Amberlite Grade 120 [21]. To ensure the uniform distribution of resin, it was poured in the form of slurry (made by mixing with deionized water) with constant stirring which prevented the air from trapping inside the column. The resin absorbed amino acids by exchanging the protons of Sulphonic acid (the activation group) having no effect on sugars [21]. Column was allowed to stand for 5 hours after adding 3ml of concentrated extract. After that the rest of the sample was subjected for separation. The sugars eluted along with deionized water at the flow rate of 250ml/hour. Elutes were collected in five different fractions of 100ml each. All the fractions were subjected to Molisch test [20] and amino acid detection tests to determine the presence of sugars and amino acids. The fractions that showed positive test for sugars and negative test for amino acids were stored and were further investigated. For the detection of total sugars and reducing sugars all the sugar containing fractions were mixed together. From the mixed sugars fraction 1ml was taken and was used for the estimation of total sugars by Anthrone method [22]. The reducing sugars were estimated by following the potassium ferricyanide method [23]. The rest of the mixed sugar fraction was put in the oven at 40-45^{0}C, the slow evaporation of this fraction led to dryness and a solid substance is obtained. This solid substance was dissolved in 0.5ml of 75%ethyl alcohol and was stored for further analysis.

Qualitative Analysis of Sugars: Ascending Paper Ion Exchange Chromatography was used for the separation and identification of sugars. Whatmann no. 1 having size of 46 x 57cm was loaded with named samples that were S_a, S_b, S_c, S_d, S_e, S_f, S_g, S_h, S_i and standard sugars. 7:1:2 was the ratio for the solvent system consisted of isopropanol, butanol and water. 48 hours were given for the development of chromatogram. The developed chromatogram was allowed to dry in the air and then was sprayed with the solutions of aniline-phthalate. The chromatogram was dried again in the air and then kept in the oven at 110- 120^{0}C for 10-15 minutes. After that R_f value of each spot was measured and the spots were compared with standard sugars' spots. The known and unknown sugars were marked.

Table 1: Types of Sugars in the Mangnefira Indica Variety Langra

Standard sugars	R_f Values x 100	Healthy Plant			Adjacent to diseased Plant			Diseased Plant		
		S_a	S_b	S_c	S_d	S_e	S_f	S_g	S_h	S_i
U*	-	-	-	-	-	-	-	-	-	78
Xylose	75	-	-	-	-	-	-	-	-	72
Fructose + Arabinose	68, 69	65	65	65	-	66	64	-	-	-
Glucose	60	59	58	59	-	-	57	-	-	-
Lactose	29	-	-	-	-	-	-	31	32	-

U* is an unknown sugar

Table 2: Total, Reducing and Non-reducing Sugars concentration in Mango Variety Langra in g/100g

Parameters	Healthy Bark	Adjacent to Diseased Bark	Diseased Bark
Total Sugars	5.58 ± 1.27	2.60 ± 0.3	3.59 ± 0.119
Reducing Sugars	2.81 ± 0.65	1.11 ± 0.36	2.36 ± 0.28
Non-reducing sugars	2.77 ± 1.146	1.48 ± 0.19	1.24 ± 0.37

Quantitative analysis of Total sugars: Method by Traveyan and Harroson [22] was used to quantify the concentration of total sugar content in the samples. To prepare a standard graph, stock solution of glucose in 0.1ml, 0.2ml…1.0ml was taken in separate test tubes. The volume of each solution was made up to 1ml by adding water followed by the addition of 5 ml of Anthrone. The test tubes were swirled well and then were covered by the glass balls and placed in the hot water for approximate 20 minutes. They were cooled off immediately by running under the tap water. Blue green color in each case was observed. Shimadzu UV120-01 spectrophotometer was used as a reference to record the absorbance at 620nm against Anthrone reagent prepared in Sulfuric acid. The graph showing the galactose standard was obtained by plotting the absorbance against the known amount of galactose. The prepared samples were treated with the similar procedure and the absorbance at 620nm was recorded. The standard graphs were used then to calculate the total amount of sugar in 1g of sample.

Estimation of Reducing sugars: Method by Hulme and Narain [23] was followed to estimate the reducing sugars in the samples. Each sample was taken in a conical flask which is then mixed with the solution of sodium carbonate and potassium ferricyanide, 2ml each. Water was added in the mixture to make the total volume up to 14ml. The mixture was heated in the similar fashion as in previous case for 15 minutes and then cool down. Then 3ml of Potassium iodide-Zinc sulfate-sodium chloride solutions was added to each flask followed by addition of 2ml of sulfuric acid solution.

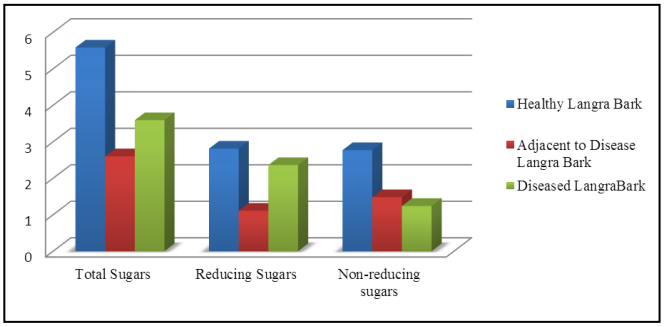

Fig 1: Sugar Content in Healthy, Adjacent to Disease and Diseased Mango Variety Langra

Couple of drops of Starch was used as an indicator. The mixture was then titrated against sodium thiosulfate solution preceding a colorless endpoint. Hulme and Narain formula was used to calculate the total reducing sugar

$$S = b\,(T + a)$$

Where,
S= amount of sugars
b= a terminal factor of 0.34
T= volume of sodium thiosulfate used
a= a terminal factor of 0.05

Non- reducing sugars were calculated by difference:
Non- reducing sugar = Total sugar - reducing sugar

3. Results and Discussion

The impact of Quick decline on the sugar contents in adjacent to disease and diseased bark of Mango variety Langra was analyzed in comparison to sugar contents in bark of healthy Mango variety Langra. The difference was studied both qualitatively and quantitatively. The results showed that significant change in sugar contents occur when the mango plant gets infected by Quick Decline.

Total six sugars were spotted in the chromatogram namely, Xylose, Fructose, Arabinose, Glucose, Lactose and an unidentified sugar. The three sugars viz. Fructose, Arabinose and Glucose were present in the samples of healthy and adjacent to disease bark with a non-uniform distribution (S_a – S_f). Interestingly, xylose, lactose and an unidentified sugar were present in the samples (S_g– S_i) of diseased bark and were absent in samples of healthy and adjacent to disease barks. The unidentified sugar was not further studied.

A significant decrease in concentration of the sugar contents in adjacent to disease and diseased barks has been noted. This quantitative analysis of total, reducing and non-reducing sugars supports the presence and absence of certain sugars in the different samples as displayed in the Table no. 1.

The sudden decrease in concentration of reducing sugars in bark samples of adjacent to disease as compared to sugar content in bark samples of healthy plant which slightly elevated in the diseased plant is noteworthy. On the other hand, the decrease in concentration of non-reducing sugars remained uniform. This may points out towards the mechanism of change in sugar content in the lifecycle of a mango plant variety Langra. Similar relationship was noted by Shad *et al* [2] and Shaheen *et al* [5] in their investigation of sugar contents in leaves of healthy and diseased mango plant variety Langra in relation to quick decline.

From the perspective that in a healthy ripened fruit the concentration of total sugars attributes to the extent of ripening [24] whereas the results of this study indicates that Quick decline also affects the fruit production of the plant by disturbing the sugar metabolism of the plant. In other words, the pathogens contribute to destructive sugar metabolism in diseased Mango plant. Also, the change in sugar content may be result of plant's defense mechanism [5]. The decrease in sugar content may also characterize the disturbed anabolism such as photosynthesis [2].

4. Conclusion

This research addressed the qualitative and quantitative changes that occur in sugar contents of Mango plant variety Langra in relation to Quick decline. Mango production has been greatly affected worldwide due to the persisting and destructive effects of Quick Decline resulting in economic and food sources' loss. Significant differences in concentration and types of sugar contents in samples of healthy, adjacent to disease and diseased came across in the results. Also, an unidentified sugar also appeared in the sample (S_i) of diseased mango plant which can be further investigated to consider pathogenic metabolism of Quick Decline in Mango Plant Variety Langra. Thus, this research will be helpful in pursuing deeper understanding of sugar metabolism in lifecycle of Mango Plant variety Langra in relation to Quick Decline.

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