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Biochemical alterations in healthy and infected stem bark of mango genotypes infected by black banded disease

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Abstract
In this study, the level of total sugars, total phenols and total alkaloids were studied in the healthy and infected stem bark of six genotypes. Biochemical analysis of healthy and infected bark of mango showed that there was considerable decrease in the reducing sugar, non-reducing sugar, total sugars and total alkaloids content of infected bark. While phenol content of the infected bark showed considerable increase as compared to the healthy bark. In the present study, the contents of total sugars, reducing sugars and non-reducing sugars were higher in healthy bark of all the genotypes and decreased in infected bark.

Keywords: Mango, Black band, biochemical, sugars, phenols

Introduction
The mango (*Mangifera indica* L.) belongs to family Anacardiaceae, originated in South East Asia at an early date. It is the most popular fruit crop in India as well as in tropical and subtropical countries of the world. Mango which is considered to have been originated from Indo-Burma region, is the most popular fruit in India and graded to be the choicest of all indigenous fruits. However, it suffers from a number of diseases at all stages of its development *i.e.*, right from nursery stage to grown up plants and even fruits at pre and post harvest stages are affected making them unsuitable for marketing and export.

The disease reaction has been correlated with the sugar level in different crop plants. Generally high levels of total sugars, reducing sugars and non-reducing sugars in the host plant were stated to be responsible for disease resistance (Bateman and Millar, 1966) [1]. The reduction in carbohydrate contents after infection may be due to rapid hydrolysis of sugars during pathogenesis through enzymes secreted by pathogens and subsequent utilization by pathogens for their development (Jayapal and Mahadevan, 1968) [5]. In general, an increased level of phenols was observed in infected leaf tissues compared to healthy tissues (Petkovsek *et al.*, 2014) [9]. Phenolic compounds slow down fungal growth, reacting with proteins and causing a loss of enzymatic function. Moreover, they restrict the viability of pathogens and deposited inside the cell wall as an important first line of defense against fungal penetration and infection (Schwalb and Feucht, 1999) [11]. A large family of N containing secondary metabolites found in approximately 20 per cent of the species of vascular plants (Hegnauer *et al.*, 1988) [4], most frequently in the herbaceous dicot and relatively a few in monocots and gymnosperms. Generally, most of them, including the pyrrolizidine alkaloids (PAs) are toxic to some degree and appear to serve primarily in defense against microbial infection and herbivore attack.

Material and Methods
Plant material collection
Both healthy and infected branches of mango tree of six genotypes namely Alphonso, Kesar, Mallika, Neelum, Raspuri and Totapuri were collected for estimation of total sugars, phenols and alkaloids.

Extraction of plant tissue in alcohol for sugar and phenol estimation
Estimation of metabolites requires their complete extraction from tissues, for this activities of the enzymes which synthesize and utilize them need to be stopped at once to get reliable values. Though water is universal solvent, it does not penetrate tissues quickly enough to stop enzymatic activity.

In this context, alcohol especially warm alcohol is the choicest solvent for extraction. The bark samples for the estimation of sugar and phenols were crushed using liquid nitrogen to get fine powder. One gram of finely powdered tissue was homogenised thoroughly with 10 ml of hot (65 ˚C) 80 per cent alcohol using mortar and pestle (Sadasivam and Manikam, 1992) [10]. The extract was filtered through double-layered muslin cloth and the residue was re-extracted following the above procedure. The filtrates obtained from both the extractions were filtered through Whatman No.1 filter paper and the dark green coloured filtrate was clarified (removal of pigments) and stored at 4 ˚C for further analysis.

Clarification of alcoholic extracts

Dark coloured alcohol extracts of the tissues create a great problem in analytical procedure. Hence, heavy metal salts were used for clarification of alcoholic extracts. Two ml of lead acetate solution was added drop wise to coloured alcoholic extract followed by 3 ml of saturated solution of disodium hydrogen phosphate till the precipitation is completed. The above solutions were mixed thoroughly, kept overnight, filtered through Whatman No. 1 filter paper and filtrate was made up to 10 ml with 80 per cent alcohol. The alcoholic extract obtained was stored in refrigerator at 4 ˚C for further use. By using this extract reducing sugars, non-reducing sugars, total sugars and total phenols were estimated in both healthy and infected bark material.

Estimation of reducing sugar

Sugars by virtue of the presence of free or potentially free aldehyde or keto groups in them reduce certain metallic ions such as copper, bismuth, mercury, iron and silver in alkaline solution medium. The colour change is quantified based on the amount of sugar present. The intensity of the colour is directly proportional to the amount of reducing sugar present in the sample. Reducing sugars were estimated by Nelson-Somogyi's method (Norton Nelson, 1944) [6].

Acid hydrolysis of non-reducing sugar and its estimation as reducing sugar

Non-reducing sugar was first hydrolysed with the help of diluted Hydrochloric acid (HCl). The hydrolysis was neutralised with a suitable alkali and the reducing sugar was estimated by Nelson-Somogyi's method (Norton Nelson, 1944). The reducing sugar in the hydrolysate was a measure of total sugar. To get the non-reducing sugar, the quantity of reducing sugar was subtracted from that of total sugar. Total sugars, reducing sugars and non-reducing sugars were presented as mg/g dry weight of tissue.

Estimation of Total phenols

Estimation of total phenols present in mango bark was done following Folin- Ciocalteau reagent (FCR) method (Bray and Thorpe, 1954) [2]. Estimation of phenols by Folin-Ciocalteau (FCR) is based on the reaction between phenols and an oxidizing agent, phosphomolybdo-phosphotungstate (FCR) which results in the formation of blue coloured complex with λ max at 660 nm. The intensity of the colour is directly proportional to the amount of phenol present in the sample.

Total alkaloids estimation

Preparation of plant extracts for estimation of alkaloids

Five gram of powdered plant material was taken into 20 ml of n-butanol and vigorously stirred. The content was transferred into a reagent bottle. The slurry was kept overnight at room temperature. Then it was centrifuged at 6000 rpm for 10 min and the supernatant was made up to 50 ml with n-butanol.

Determination of total alkaloids by titrimetric method

Supernatant obtained from plant sample was used for the determination of total alkaloids by titrimetric methods. 10 ml of the supernatant was taken into a 100 ml separating funnel. 10 ml of 0.1 N HCl was added and shaken thoroughly for 2-3 min. This resulted in the solubility of alkaloids. The lower layer contained alkaloids neutralized with 0.1 N HCl and the upper layer contained n-butanol. 10 ml HCl portion was collected in a beaker and 2-3 drops of methyl red was added to it, that turns the solution into slightly reddish colour. The contents of beaker were titrated against 0.1 N NaOH, till colour changed from red to pale yellow. The neutralization point was determined. Same procedure was repeated thrice.
The total amount of alkaloids was calculated by considering the following equivalent:

1 ml of 0.1 N HCl = 0.0162 g of alkaloid

Results

The total sugars, reducing sugars and non-reducing sugars recorded in healthy and infected mango bark of different genotypes is presented in (Table 1).

Total sugars

The results revealed that there was a decrease in total sugar content from healthy to infected bark and this differed significantly among genotypes and treatments (bark condition *i.e.*, Healthy and infected). Among the treatments the maximum mean total sugar content was recorded in healthy bark (1.64 mg/g dry weight) and the minimum total sugar content was recorded in infected bark (1.36 mg/g dry weight). Among the genotypes the maximum mean total sugar content was recorded in Kesar (2.11 mg/g dry weight) and the minimum in case of Totapuri (1.06 mg/g dry weight).
Both healthy (2.23 mg/g dry weight) and infected (2.0 mg/g dry weight) bark of Kesar genotype contained highest content of total sugars. This was followed by Alphonso in healthy (1.84 mg/g dry weight) and in infected condition (1.31 mg/g dry weight). The least total sugar content was noticed in Totapuri in both healthy and infected condition (1.21 mg/g dry weight and 0.91 mg/g dry weight, respectively).
Alphonso recorded maximum per cent decrease of total sugar (28.80%), followed by Totapuri (24.79%). Whereas, Mallika recorded the least per cent decrease of total sugar (9.15%).

Reducing sugars

As seen from the Table 1, the reducing sugar content varied significantly among different genotypes and treatments (healthy and infected bark condition). The highest mean reducing sugar content was recorded in healthy bark (1.16 mg/g dry weight) and the least reducing sugar content was recorded in infected bark (0.96 mg/g dry weight). Among the genotypes, the highest mean reducing sugar was recorded in Kesar (1.65 mg/g dry weight) and the least in case of Totapuri (0.73 mg/g dry weight).
Among different genotypes, maximum reducing sugar content was recorded in Kesar in both healthy (1.74 mg/g dry weight) and infected bark (1.57 mg/g dry weight), followed by Alphonso healthy bark had 1.34 mg/g dry weight and infected bark had (0.92 mg/g dry weight) which is on par with the infected condition in Raspuri (0.92 mg/g dry weight) genotype. The least reducing sugar content was recorded in

Totapuri in both healthy and infected bark (0.83 and 0.64 mg/g dry weight, respectively).
The per cent decrease of reducing sugar content varied among genotypes. The per cent decrease of reducing sugar content was maximum in Alphonso (30.56%), followed by Totapuri (22.89%) and minimum in case of Mallika (8.88%).

Table 1: Effect of black banded disease on accumulation of total sugars in bark of different genotypes of mango

Sl. No.	Genotype	Total sugars (mg/g dry weight)				Reducing sugars (mg/g dry weight)				Non-reducing sugars (mg/g dry weight)			
		Healthy	Infected	Mean	Per cent decrease over healthy	Healthy	Infected	Mean	Per cent decrease over healthy	Healthy	Infected	Mean	Per cent decrease over healthy
1	Alphonso	1.84	1.31	1.57	28.80	1.32	0.92	1.12	30.56	0.52	0.39	0.45	24.38
2	Kesar	2.23	2.00	2.11	10.31	1.74	1.57	1.65	09.77	0.49	0.43	0.46	12.24
3	Mallika	1.42	1.29	1.35	09.15	0.90	0.82	0.86	08.88	0.52	0.47	0.49	09.61
4	Neelum	1.52	1.31	1.41	13.82	1.07	0.89	0.98	16.52	0.45	0.42	0.43	07.47
5	Raspuri	1.63	1.36	1.49	16.56	1.12	0.92	1.02	17.56	0.51	0.44	0.47	13.53
6	Totapuri	1.21	0.91	1.06	24.79	0.83	0.64	0.73	22.89	0.38	0.27	0.32	28.95
Mean		1.64	1.36	1.49	17.23	1.16	0.96	1.06	17.69	0.47	0.40	0.43	16.03
Source		S. Em.±		C. D. at 1%		S. Em. ±		C. D. at 1%		S. Em.±		C. D. at 1%	
Genotype (G)		0.02		0.07		0.02		0.08		0.01		0.04	
Treatment (T)		0.01		0.04		0.01		0.04		0.01		0.02	
G × T		0.03		0.10		0.03		0.11		0.01		0.05	

Non-reducing sugars
The results revealed that there was a decrease in non-reducing sugar content from healthy to infected bark and it differed significantly among genotypes and treatments (bark condition *i.e.*, Healthy and infected). The maximum mean non-reducing sugar content was recorded in healthy bark (0.47 mg/g dry weight) and the least non-reducing sugar content was recorded in infected bark (0.40 mg/g dry weight). Among the genotypes, the highest mean non-reducing sugar was recorded in Mallika (0.49 mg/g dry weight) and the least was recorded in Totapuri (0.32 mg/g dry weight).
The data showed that among different genotypes, maximum non-reducing sugar content was recorded in healthy condition of Alphonso and Mallika (0.52 mg/g dry weight) Raspuri (0.51 mg/g dry weight) these three were on par with each other, this was followed by Kesar (0.49 mg/g dry weight) and the least was recorded in Totapuri (0.38 mg/g dry weight). In infected condition maximum non-reducing sugar content was recorded in Mallika (0.47 mg/g dry weight) followed by Raspuri (0.44 mg/g dry weight) and the least was recorded in Totapuri (0.27 mg/g dry weight).
The per cent decrease of non-reducing sugar content varied among genotypes. The maximum per cent decrease of non-reducing sugar content was noticed in case of Totapuri (28.95%), followed by Alphonso (24.38%). The least per cent decrease of non-reducing sugar was recorded in Mallika (4.54%).

Total phenols
The total phenol content of different genotypes recorded in healthy and infected mango bark is depicted in Table 2. The results revealed that there was an increase in total phenol content from healthy to infected bark and it varied significantly among genotypes and treatments. Among the treatments the maximum mean total phenol content was recorded in infected bark (1.90 mg/g dry weight) and the minimum total phenol content was recorded in healthy bark (1.45 mg/g dry weight). Among the genotypes, the maximum mean total phenol was recorded in Mallika (2.04 mg/g dry weight) and the least in case of Raspuri (1.52 mg/g dry weight). Mallika recorded maximum total phenol content in both healthy (1.66 mg/g dry weight) and infected bark (2.42 mg/g dry weight), this was followed by Alphonso in healthy (1.52 mg/g dry weight) and infected bark (1.84 mg/g dry weight). The maximum per cent increase of total phenol in infected bark over healthy was recorded in Mallika (45.78%), followed by Kesar (34.78%). The least per cent increase of total phenol in infected bark over healthy was noticed in Alphonso (21.05%).

Total alkaloids
The total alkaloid content recorded in different genotypes of mango under healthy and diseased bark condition is depicted in Table 2. From the table it was observed that there was a significant difference among genotypes and treatments. The highest mean total alkaloid content was recorded in healthy bark (9.34 mg/g dry weight) and the least total alkaloid content was recorded in infected bark (7.70 mg/g dry weight). Among the genotypes, the highest mean total alkaloid was recorded in Raspuri (9.11 mg/g dry weight) and the least in case of Totapuri (8.20 mg/g dry weight).
Among the different genotypes tested in healthy condition, Raspuri recorded maximum total alkaloid content (9.88 mg/g dry weight), followed by Alphonso (9.72 mg/g dry weight) in healthy condition and the least by Neelum (8.51 mg/g dry weight). In infected condition, maximum alkaloid content was recorded in Mallika (8.50 mg/g dry weight) followed by Raspuri (8.34 mg/g dry weight) and the least was recorded in case of Totapuri (7.00 mg/g dry weight).
The per cent decrease of total alkaloid content in infected over healthy was the highest in case of Totapuri (25.50%), followed by Alphonso (25.41%). Whereas the lowest per cent decrease of total alkaloid over healthy was noticed in Mallika (10.31%)

Table 2: Effect of black banded disease on accumulation of total phenols and alkaloids content in bark of different genotypes of mango

Sl. No.	Genotype	Total phenols (mg/g dry weight)				Total alkaloids (mg/g dry weight)			
		Healthy	Infected	Mean	Per cent increase over healthy	Healthy	Infected	Mean	Per cent decrease over healthy
1	Alphonso	1.52	1.84	1.68	21.05	9.72	7.25	8.48	25.41
2	Kesar	1.38	1.86	1.62	34.78	9.07	7.91	8.49	12.81
3	Mallika	1.66	2.42	2.04	45.78	9.48	8.50	8.99	10.31
4	Neelum	1.36	1.73	1.54	27.21	8.51	7.23	7.87	14.99
5	Raspuri	1.31	1.74	1.52	32.82	9.88	8.34	9.11	15.60

6	Totapuri	1.49	1.86	1.67	24.83	9.40	7.00	8.20	25.50
Mean		1.45	1.90	1.67	31.07	9.34	7.70	8.52	17.43
Source		S. Em. ±		C. D. at 1%		S. Em. ±		C. D. at 1%	
Genotype (G)		0.01		0.06		0.02		0.07	
Treatment (T)		0.01		0.03		0.01		0.04	
G × T		0.02		0.08		0.03		0.10	

Discussion

Generally the parasite or pathogens are drawing nutrients from their host plant which leads to certain biochemical changes in the infected part of the host. The biochemical changes induced by the pathogen in the host plant help in understanding the host-pathogen relationship in many ways. In this study attempts have been made to get a preliminary idea about the biochemical changes induced by the pathogen in infected plant part in respect to total sugars, phenols and alkaloid contents. Here different genotypes with different degree of PDI were used to understand the host- pathogen relationship.

Total sugars

The primary metabolites include carbohydrates, which are exploited by the pathogen for their growth and development. Generally high levels of total sugars, reducing sugars and non-reducing sugars in the host plant were stated to be responsible for disease resistance (Jayapal and Mahadevan, 1968) [5].

In the present study, the contents of total sugars, reducing sugars and non-reducing sugars were higher in healthy bark of all the genotypes and decreased in infected bark. But the quantity of these sugars was higher in genotypes which had low disease incidence and severity. This clearly suggests that the low level of incidence and severity may be one of the reasons because of higher quantity of all kinds of sugars. The decreased level of sugars in infected tissues of all genotypes may be due to variation in the extent of incidence and severity and also because of the utilization of sugars for the growth and development of the pathogen and also conversion of sugars into simpler molecules during pathogenesis through enzymes secreted by pathogens. Similar observations were made by Patil and Dangat (2011) [7] and Chirag (2015) [3] who reported that there was a considerable decrease in the contents of reducing, non-reducing and total sugars contents of infected bark as compared to healthy bark.

Total phenols

Phenols have been found to play an important role in determining resistance or susceptibility of a host to parasitic infection. High concentration causes an instant lethal action by a general tanning effect while, low concentration causes gradual effect on cellular constituent of the parasite. If the concentration does not occur at toxic level, the inhibition will be obviously slow. Besides, the pathogen readily detoxifies low concentrations of toxicants rather than high concentrations.

In the present study, lower levels of phenols were observed in healthy bark and higher levels in infected bark of all the genotypes tested. In infected bark their amount increased in all genotypes, but this increase in phenol content was at higher rates in genotypes with lower incidence and severity while it was at lower rates in genotypes of higher incidence and severity. In Alphonso it was 21.05 per cent and in Mallika it was 45.78 per cent. Phenols are produced and accumulated at a faster rate after infection at the site of infection and they inhibit the production of fungal enzymes that cause degradation of host tissue and also phenols act as precursor for lignin. Therefore, per cent disease incidence and severity was found to be less in genotypes with higher amount of phenols in their infected tissue and severity was higher in case of genotypes with lower amounts of phenol in their infected tissue as compared to healthy tissue. This probably may be speculated that total phenol content in the bark imparts resistance against the disease. The results of the experiment are in line with the Patil and Dangat (2011) [7] and Chirag (2015) [3]. They reported that phenol content of the infected bark increased as compared to that in healthy bark.

Total alkaloids

Generally, alkaloids are extremely toxic though they have a marked therapeutic effect in small quantities. They ensure plant survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrence) (Pearce *et al.*, 1991) [8].

The results in the present investigation revealed that total alkaloid content was maximum in healthy bark as compared to infected. This might be due to degradation of plant cell wall and membrane by pathogens which leads to loss of alkaloids, as these alkaloids are volatile in nature. Therefore, alkaloid content was reduced in infected bark. There are no supporting references for this study, which needs confirmation by taking some more genotypes and more number of samples.

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