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Changes in chemical constituent of Custard apple associated with rot by *Phoma*

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ABSTRACT

Biochemical changes in the custard apple, *Annona squamosa* L. as a result of infection by *Phoma* sp. were investigated in regard to protein profiles, saccharides and ascorbic acid levels. The protein level showed a gradual increase at the initial stages of infection which on progress of infection, its level gradually declined to a minimum as compared to the healthy ones. The non-reducing sugar exhibited significant decrease as the disease proceeded progressively while ascorbic acid content was found to decline gradually to a minimum in the host tissues following infection. The resultants of infection lead to blackening of the pulp of the fruit, which ultimately sheds off.

Key words : *Phoma* sp., *Annona squamosa*, Reducing sugar, Ascorbic acid.

Custard apple or sugar apple (*Annona squamosa* Linn.) belonging to the family Annonaceae is one of the best tropical fruits, which enjoys a high demand due to agreeable sweetness with a most delightful fragrance. Fruit rot caused by *Phoma* sp is most common epiphytotic dreadful disease of custard-apple causing yield-loss. Biochemical changes in the host tissues as a result of infection by rot fungi; thereby altering the nutritional values of the fruit tissues have been studies by several workers (Nema, 1989; Ram and Vir, 1996; Dutta and Chatterjee, 2002). The present communication has been designed to study the post-infectional biochemical changes in different nutritional parameters of diseased tissue of custard apple.

MATERIALS AND METHODS

The organism associated with infected fruits was isolated and identified as *Phoma* sp. The identification of the pathogen (ITCC No. 5864.04) has been confirmed by Indian type culture collection, IARI, New Delhi.

Total carbohydrate content was estimated quantitatively following the method of McCready *et al*. (1950) after suitable modifications. 1g of fresh host tissues of each healthy and infected custard apple was homogenised and extracted with hot 80% ethanol maintaining the volume at 10 ml. The extracted ethanolic solution was made colourless with activated charcoal and filtered. The residual plant tissues were treated with 10 ml of 70% perchloric acid to obtain the extract of starch. Finally, the ethanolic extract and acid extract were made upto the volume of 100 ml separately with the addition of distilled water.

One ml of aliquot was taken separately from these two extracts and to each of these aliquots, 4ml of 0.1% anthrone reagenet (100mg anthrone in 100ml of conc. H_2SO_4) 4h to 9 days old, was rapidly added, mixed thoroughly with a glass rod and allowed to cool. The tube was placed in a bath of boiling water for 10 min by placing marbel on the top of the test tubes to prevent the loss of water evaporation followed at 620 nm against a reagent blank in a spectrophotometer. The blank set contained 1 ml of distilled water instead of 1ml of aliquot.

Protein content was measured following the method described by Bradford (1976) with Bradford's reagent using Coomassie Brilliant Blue (Fluka). For estimation of total protein, healthy and infected dry fruit tissues (20mg) were crushed separately with 2 to 2.5 ml of methanol with a pinch of neutral sand and centrifuged at 5000 rpm for 15 min. The pellet was taken discarding the supernatant. The pellet added with 2.5 ml of 1(M) NaOH was kept at 80°C for 1h and cooled at room temperature. The volume was adjusted to the initial level and centrifuged at 10,000 rpm for 15 min. The supernatant served as protein source. To 0.1ml of the supernatant, 5ml of Bradford reagent was added after 2 but within 10 min, the absorbance was measured spectrophotometrically (Beckman Model) at 595 nm against a reagent blank prepared from 0.1 ml of appropriate buffer and 5ml of Bradford's reagent.

Ascorbic acid was estimated by the method described by Oser (1979). Aliquots of 100mg fresh tissues of both healthy and infected fruit were homogenised separately with 5ml of 60% trichloroaceticacid (TCA). After centrifugation at 5000 rpm for 5 min, the supernatant was collected and a pinch of activated charcoal (Norit) was added to it and filtered. The volume of the filtrate was made up to 100ml with distilled water. An aliquot of 4ml was taken in the test tube and 2ml of 2% 2, 4-dinitrophenyl hydrazine [2g of 2, 4-dinitrophenyl hydrazine in 100ml of 9(N) H_2SO_4] and one drop of 10% thiourea solution (in 70% alcohol) was added to it. The mixture was then boiled for 15 min in a water bath followed by cooling at room temperature. Then 5ml of 80% H_2SO_4