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Research Article

The Effect of Early Blight Disease and Biochemical Changes in Tomato Crop

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Abstract

Biochemical changes were observed in healthy as well as infected tomato leaves and fruits caused by *Alternaria solani*. There was a significant variation between healthy and infected leaves and fruits which showed significant changes with respect to estimation of lycopene, protein, phenol, ascorbic acid, total sugar and chlorophyll. Lycopene content in US-2175 variety was decreased due to *Alternaria solani* while, protein content in US-618, US-2175; ascorbic acid content in SBGI-555, Swadeshi and Veer variety, phenol content in SBGI-555 variety; total sugar content in Veer variety and Chlorophyll content in leaf of Bioseed-56 variety was drastically hampered due to *Alternaria solani*.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) belongs to the family solanaceae and is one of the most remunerable and widely grown vegetables in the world. Among the vegetables tomato ranks next to potato in world acreage and ranks first among the processing crops. Tomato is grown for its edible fruits, which can be consumed either fresh or in processed form and is a very good source of vitamins A, B, C and minerals. Tomato cultivation has become more popular since mid nineteenth century because of its varied climatic adaptability and high nutritive value. Tomato is being exported in the form of whole fruits, paste and in canned form to West Asian countries, U.K., Canada and USA. There are several diseases on tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992). Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the world's most catastrophic disease. The causal organism is air borne and soil inhabiting and is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981). The disease appears on leaves, stems, petiole, twig and fruits under favourable conditions resulting in defoliation, drying off of twigs and premature fruit drop and thus causing loss from 50 to 86 percent in fruit yield (Mathur and Shekhawat, 1986). Pathogen also causes leaf and fruit rot in pre harvest and post harvest stages. Leaf rot causes decrease in the photosynthesis rate which ultimately causes the less synthesis of food and affect on the yield. Infected fruits are disqualified in the market. Considering this fact present investigation has been undertaken to understand biochemical changes in tomato leaves and fruit due to early blight disease.

MATERIALS AND METHODS

Changes in lycopene content

Extraction method was performed according to Fish *et al.*, (2002). Samples were first chopped and homogenized in a laboratory homogenizer. Approximately 0.3 to 0.6 g samples were weighed and 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol and 10 mL of hexane were added. The recipient was introduced in ice and stirred on a

magnetic stirring plate for 15 min. After shaking, 3 mL of deionized water were added to each vial and the samples were shaken for 5 min on ice. Samples were then left at room temperature for 5 min to allow the separation of both phases. The absorbance of the hexane layer (upper layer) was measured in a 1-cm-path-length quartz cuvette at 503 nm blanked with hexane.

Changes in protein content

Changes in protein content were estimated by using Lowry's method (1951).

(Reagents: A. 2% Na_2CO_3 in 0.1 N NaOH; B. 1% sodium pottasium tartrate in H_2O; C. 0.5% $CuSO_4$.5 H_2O in H_2O; D. 48 ml of A, 1 ml of B, 1 ml C; E. Phenol Reagent - 1 part Folin-Phenol [2N] : 1 part water; BSA Standard – 50mg BSA in 50ml D.W.).

0.2, 0.4, 0.6, 0.8 and 1 ml of working standard BSA was pipetted out in a series of test tubes. 0.1 ml of sample extract was pipetted out in another test tube. In all test tubes volume of 1 ml was made and tube with 1 ml of water served as a control. Then 5 ml of reagent C was added in all the test tubes including blank. It was then mixed well and incubated for 10 minutes at room temperature. 0.5 ml of dilute Folin-phenol solution was added to each tube. Each tube was vortexed immediately and incubated at room temperature for 30 minutes. Blue colour was appeared and at 660 nm O.D. was taken. Absorbance *vs* mg protein graph was plotted to obtain standard curve.

Changes in total sugar content

The sugar content in the leaf powder was estimated by the procedure recommended by Oser (1979) as follows.

500mg of leaf powder was taken in 50ml distilled water and boiled, then filtered. Further filtrate was diluted up to 100ml. Three Folin-wu tubes were taken and to it following content were added

(1) Blank tube - Distilled Water 2ml (2) 2ml glucose 'C' solution. (3) 2ml filtrate. In each tube 3ml alkaline solution of copper was added. Then tube was boiled in boiling water bath for 8 minutes. The tubes were cooled under tap water and 2ml of phosphomolybdic acid solution was added which gave blue colour. Then this solution was diluted up to 25ml distilled water and optical density was determined at 420nm and the amount of reducing sugar present in leaf powder was calculated.

Percent total sugar was calculated by following formula:

$$\text{Mg sugars/100mg samples} = \frac{\text{O.D. of unknown} \times 100 \times 0.4}{\text{Conc. from graph} \times 2 \times W}$$

Where, V = volume of the filtrate
W = weight of the sample taken

Changes in ascorbic acid

Vitamin C content was estimated by standard titration method. 5 ml of standard solution of standard ascorbic acid (100mg /ml) was pipette out into a conical flask, then 10ml of 0.4 % oxalic acid was taken and it was titrated with dye solution. After that 2gm sample was extracted in 0.4% oxalic acid and volume was made up to 100ml by 0.4% oxalic acid. From that solution 5ml of sample was pipette out into conical flask and titrated with dye solution. End point was pink colour. Finally amount of vitamin C in mg / 100ml pulp was estimated by using following formula.

$$\frac{\text{Amount of ascorbic acid mg}}{\text{100ml pulp}} = 0.5\text{mg}/V_1 \text{ ml} \times \frac{V_2\text{ml}/5\text{ml} \times 100\text{ml}/\text{wt. of sample} \times 100}{}$$

Where, V1 ml = volume of standard ascorbic acid.
V2ml = volume of sample's ascorbic acid.

Changes in Phenol content

The total phenolic content of leaves was determined according to the method described by Malik and Singh (1980).

Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Leaf chlorophyll assay

Healthy and infected tomato leaves were washed with sterile distilled water and Later on O.D. (Chl-a at 645nm and Chl-b at 663nm) of each sample was taken and chlorophyll content was estimated.

EXPERIMENTAL RESULTS

Table 1(a): Biochemical changes in different varieties of tomato infected with *A. solani*

Parameters	Varieties									
	Bioseed-56		Atal		Mahaveer		Karan		Veer	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Lycopene	4450.3 µg/ml	3702.4 µg/ml	4250.2 µg/ml	3527.5 µg/ml	4920.1 µg/ml	3805.5 µg/ml	4305.2 µg/ml	3909.2 µg/ml	4920.2 µg/ml	2750.2 µg/ml
Protien	65%	46%	70%	30%	67%	35%	71%	40%	69%	50%
Ascorbic acid	4750.2 µg/ml	2370 µg/ml	4450.5 µg/ml	2470 µg/ml	4570.5 µg/ml	2270 µg/ml	4650.5 µg/ml	2350 µg/ml	4020.2 µg/ml	2025.2 µg/ml
Phenols	34%	23%	35%	24%	37%	32%	38%	30%	36%	28%
Total sugar	50.0%	30.0%	52.2%	27.1%	49.5%	24.2%	48.4%	23.0%	46.9%	21.8%
Chloro-phyll	2.5	1	2.3	1.1	2.7	1.2	3.1	1.6	2.4	1.1

Table 1(b): Biochemical changes in different varieties of tomato infected with *A. solani*

Parameters	SBGI-555		Swadeshi		US-618		US-1196		US-2175	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Lycopene	4845.3 µg/ml	3334.2 µg/ml	4435.8 µg/ml	3730.8 µg/ml	4725.1 µg/ml	3600.5 µg/ml	4405.2 µg/ml	4009.2 µg/ml	4720.2 µg/ml	2800.2 µg/ml
Protien	50%	35%	33%	15%	60%	10%	70%	50%	60%	10%
Ascorbic acid	4020.2 µg/ml	1525.2 µg/ml	4520 µg/ml	1722 µg/ml	4522 µg/ml	1822 µg/ml	4270 µg/ml	2370 µg/ml	5025 µg/ml	2402 µg/ml
Phenols	23.2%	16.5%	20.2%	15.2%	21%	14.2%	24.2%	15.2%	23%	16%
Total sugar	52.2%	28.2%	50.2%	30.2%	48.2%	22%	50.2%	20.2%	45%	20%
Chlorophyll	1.9	1	2.1	1.2	2.2	1.3	2.4	1.2	1.8	0.9

Biochemical changes in different tomato varieties were calculated by using standard methods and results are given in table 1(a) and 1(b).

Lycopene content in tomato fruit were calculated. *Alternaria solani* was responsible in drastic decrease in lycopene content in US-2175 variety which is followed by Veer and SBGI-555 varieties of tomato. Protein content in infected tomato leaves were estimated by Lowery's method and results are given in table 1(a) and (1). Maximum decrease in protein content due to *Alternaria solani* was observed in US-618, US-2175, Atal and Mahaveer. Ascorbic acid content in SBGI-555, Swadeshi, Veer and US-618 tomato varieties was found to be hampered due to *Alternaria solani.* Phenol content in SBGI-555 variety leaf was drastically hampered due to *Alternaria solani* which is followed by US-618, US-2175, Bioseed-56 and Atal tomato varieities. Veer tomato variety showed maximum decrease in total sugar content in leaf due to *Alternaria solani* which is flowed by Bioseed-56, Karan, Mahaveer, SBGI-555, US-618 whereas maximum decrease in Chlorophyll content in leaf of Bioseed-56 variety was observed due to *Alternaria solani* which is followed by Veer, Mahaveer, Swadeshi and US-1196.

DISCUSSION

Spoilage means any change in the condition of food in which the food becomes less durable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture (Akinmusire, 2011). F rom results it is clear that lycopene content in tomato fruit, protein content, vitamin C content, total sugar content, phenol content and chlorophyll content in tomato leaves were found to be decrease due to *Alternaria solani*.

Similar results were reported by Ogaraku *et al.,* (2010). They found that, *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Alternaria solani and Fusarium oxysporium* hampered the vitamin C contents in tomato fruit. Aulakh and Grover (1970) reported that *Phoma destructiva* depleted the vitamin C and carbohydrate contents in tomato fruit. Loss in amount of glucose in fruits have been reported for tomato-*Drechslera australiense* (Kapoor and Tandon, 1970); tomato-*Alternaria solani* (Mehta *et al.*, 1975); banana-*Gloeosporium musarum* (Wang, 1960). On the other hand, Tandon (1970), Pandey *et al*., (1974), Fush *et al*., (1980), Reddy and Laxminarayana, (1984) and

Gadgile (2011) found that there is decrease in total sugar of mango fruit due to infection of *A. niger*. Vitamin C content of mango fruit was depleted by *Phomopsis mangiferae* and *Phoma exigua* (Reddy and Laximinarayan, 1984). Similarly, Arya (1993) reported the mango fruit infected with *Botryodiplodia theobromae* showed decrease in vitamin C content. In conclusion, the loss of vitamin C during pathogenesis may be due to production of suitable ascorbic acid degrading enzymes either by the fungus or by host pathogen interaction.

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