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Evaluation of biochemical changes in infected and non-infected plants of tomato with *Alternaria solani*

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Abstract

The present study was carried out to evaluate the biochemical changes occurring in infected and non infected leaves of tomato with *Alternaria solani*. Various biochemical constituents were studied viz., chlorophyll content (chlorophyll a and chlorophyll b), total sugars (reducing and non reducing sugar), total phenol content and total soluble protein. Results revealed that infected plants show severe necrosis and there is sudden decline in chlorophyll content (chlorophyll a and chlorophyll b) and in amount of total soluble sugar (reducing and non reducing sugar) and increase in protein and phenol content in infected plants. The result indicate that pathogen *Alternaria solani* causes severe alteration in biochemical constituents of tomato plant that directly or indirectly reduce the resistance or weakens the defense response of tomato against virulent pathogen.

Keywords: Chlorophyll content, phenol, total sugars, protein content, resistance

Introduction

Tomato (*Solanum lycopersicum* L.) is the third most important crop in India after potato and onion in terms of area and production and belongs to Solanaceae family. It is known for its high nutritive value and for its edible fruits that may be consumed either in fresh or in processed forms. It is also rich in vitamin A, B and C and minerals (Khosro, 1994) [6]. It is affected by many fungal, bacterial, viral and nematodes diseases that affect tomato production and decrease its economical value. Among the various diseases, early blight or fruit rot disease that is caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most destructive diseases of tomato that mainly occurs in the tropical as well as in subtropical regions. It causes loss both at pre and post harvest stages that leads to reduction of 35 to 78 percent in yield (Jones *et al.* 1993) [4].

Alternaria is saprophytic, endophytic and pathogenic in nature. The species of *Alternaria* are associated with a wide range of substrates that includes seeds, plants, agricultural products human being and animals. Early blight produces a wide range of symptoms at all stages of plant growth. Under favourable condition the disease appears on leaves, stems, petiole, twig and fruits resulting in defoliation, drying off of twigs and premature fruit drop (Mathur and Shekhawat, 1986) [7]. Walker (1952) [10] reported oval or angular shaped spots of 0.3 to 0.4cm diameter with usually narrow chlorotic zone around the spot. As the spots mature, concentric rings of raised and depressed brown tissue are evident. Infection accompanied by the production of toxins by *A. solani*, including some non-host specific toxins called alternaric acid, zinniol, altersolanol and macrosporin. The toxins mainly act on the host protoplast to disturb physiological processes that sustain plant health (Agrios, 2005) [1].

The pathogen infects the plant leaves at all stages of plant growth and causes destructive necrotic symptoms that leads to yield loss. The biochemical constituents like chlorophyll content, sugars, phenol and proteins are known to play an imperative role in development of resistance or induce defense response against virulent pathogen. Alteration from normal amount leads to susceptibility towards biotrophic, hemi-biotrophic and necrotic plant pathogen. Here, the present investigation was undertaken to evaluate the biochemical changes in non infected and infected plants of tomato with *A. solani*.

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Material and Method**To study the bio-chemical changes in host plants of tomato****A) Estimation of Total sugars****Reagents**

1. Anthrone reagent (2mg/ml conc, sulphuric acid)
2. Standard glucose solution (1mg/ ml): dissolved 100 mg glucose in 100 ml distilled water.
3. Working standard solution (100 mg/ml) Dilute 10 ml standard solution to 100 ml with distilled water
4. 5N HCl

Total sugar content was determined by colorimetric method using anthrone reagent. In this method, 100 mg of sample was taken in a boiling tube and hydrolyzed it in boiling water bath for 3h with 5ml of 2.5N HCl and cooled to room temperature neutralized it with solid sodium carbonate until the effervescence ceased and made the volume to 100ml and centrifuged, collected the supernatant and took 0.5 and 1 ml aliquots for analysis, then prepared the standards by taking 0,0.2,0.4,0.6,0.8 and 1 ml of working standard and made the volume to 1 ml in all the tubes including the sample tubes by adding distilled water, after that 4ml of anthrone reagent was added, heated for 8 min in a boiling water bath, cooled it rapidly and read the green to dark green colour at 630 nm. The amount of sugars present in the sample was plotted against standard curve prepared from glucose. The sugar content in plant samples was expressed as mg g⁻¹ fresh tissue (Dubois *et al.*, 1956)^[2].

Estimation of reducing sugars**Reagents****Copper reagent "A"**

Sodium carbonate (anhydrous)	2.5 g
Potassium sodium tartrate	2.5 g
Sodium bicarbonate	2.0 g
Sodium sulphate	20.0 g
Distilled water	80.0 ml
Volume	100 ml

Copper reagent "B"

Copper sulphate	15 g
Conc. sulphuric acid	1 drop
Volume	100 ml

Alkaline copper tartrate

Copper reagent "A"	24 ml
Copper reagent "B"	1 ml

Arseno-molybdate reagent

Ammonium molybdate	2.5 g
Cone. Sulphuric acid	2.5ml
Disodium hydrogen arsenate	0.3 g
Volume	70 ml

Standard glucose solution (1 mg/ ml)	Dissolve 100 mg glucose in 100 ml of distilled water
Working standard solution (100mg/ml)	Dilute 10 ml standard solution to 100 ml of distilled water

Reducing sugar content was measured following "Nelson's modification of somogyi's method" (Somogyi, 1952)^[8] using arseno-molybdate colour forming reagent and two copper reagent "A" and "B". In this 100 mg of sample was taken and extracted the sugars with hot 80% alcohol twice, collected the supernatant and evaporated on sugar bath, added 10 ml water

and dissolved the sugars, pipetted out aliquots of 0.1 or 0.2 ml of alcohol-free extract to separate test tubes. Then pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution into a series of test tubes, made up the volume in both samples and standard tubes to 2 ml with distilled water, pipette out 2 ml distilled water into a separate tube to serve as a blank, added 1 ml of alkaline copper tartarate reagent to each tube, placed the tubes in a boiling water for 10 min, cooled the tubes and added 1 ml of arsenomolybdc acid reagent to all the tubes. Made the volume in each tube to 10 ml with water and absorbance was measured at 620 nm on Spectronic-20. The value was plotted against a standard curve prepared from glucose. The figures were expressed on percentage basis.

Estimation of Non- reducing sugar

The amount of non-reducing sugar was obtained by subtracting reducing sugar from the amount of total sugars and multiplying the resultant with a constant factor 0.95.

B) Estimation of total phenol content

The total phenol content was estimated by the method described by Thimmaiah (1999)^[9]. One gram root or shoot sample was grind in mortar and pestle with 10 ml 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered and the residue was re-extracted with five-time volume of 80 per cent ethanol, supernatant was cooled and evaporated to dryness in water bath. The residue was dissolved in 5 ml of distilled water. An aliquot of 0.2 ml was transferred in test tube and volume was made to 3 ml with distilled water, Folin-ciocalteau reagent (0.5ml) was added in each test tube. After three minutes, 2 ml of 20 per cent sodium carbonate was added in each tube and mix thoroughly. The tubes were then placed in boiling water for one minute. After cooling, the absorbance was recorded at 650 nm against a reagent blank. The standard curve was prepared by taking different concentrations of catechol. The phenol content was express as mg g⁻¹ fresh tissue.

C) Estimation of soluble protein content

The soluble protein content of the samples was assayed by using the method of Lowry *et al.* (1951)^[5]. One gram of root or shoot was macerated in mortar with 5 ml 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged of 16,000 g for 20 minutes. The supernatant was used for estimation of soluble protein content. For this purpose, two per cent sodium carbonate (anhydrous) in 0.1 N NaOH (Solution A) was prepared. Similarly, 0.5 per cent copper sulphate (CuSO₄·5H₂O) in 1 per cent sodium potassium tartarate (freshly made) was prepared (solution B). From these two reagents, solution C (alkaline copper sulphate) was prepared by mixing 50 ml of solution A with 1 ml of solution B just before use. An aliquot of 0.1 ml supernatant was taken in test tube and the volume was made to 1 ml with distilled water followed by addition of 5 ml solution C mixed well and incubated at room temperature for ten minutes. A 0.5 milliliter of folin ciocalteu reagent was diluted to 1N, mixed well and incubated at room temperature in dark for 30 minutes. The absorbance was recorded at 660 nm against blank. The amount of protein in sample was computed from the standard curve prepared by using different concentrations of bovine serum albumin. It was expressed as part per million (ppm).

D) Estimation of chlorophyll content

Total chlorophyll content was estimated by the method (Hiscox and Israelstom, 1979) [3]. Sample extract was prepared from 50 mg of leaf sample placed in 5 ml of DMSO (Dimethyl sulphoxide). These samples were heated in an incubator at 65 °C for 4 hrs and then after cooling to room temperature, the absorbance of extracts was recorded at 470, 663 and 645 nm. Chlorophyll content was calculated

$$\text{Chl a} = [12.7 \times A_{663} - 2.69 \times A_{645}]$$

$$\text{Chl b} = [22.9 \times A_{645} - 4.68 \times A_{663}]$$

$$\text{Chl}_{\text{Total}} = [20.2 \times A_{645} + 8.02 \times A_{663}]$$

The values thus obtained are in µg/ml of extract (solvent)

Results

The bio-chemical analysis of early leaf spot diseased plants and healthy plants of tomato were carried out in single variety (S-22) and comparison of bio-chemical changes in infected plants with healthy ones was done. The non structural carbohydrates *i.e.* total, reducing and non-reducing sugars, soluble protein and total phenol and chlorophyll content were estimated after 45 days of sowing of the crop. All the biochemical constituents were estimated from fresh leaves of healthy and diseased plants of tomato after 15 days of inoculation with the pathogen.

A) Effect on total soluble sugar (Reducing & Non reducing sugar)

There was significant reduction in total soluble sugar, reducing and non-reducing sugar content of infected tomato

leaves when compared with healthy ones. The total sugar content was 3.53 mg /g in healthy leaves whereas it was 2.19 mg/g in infected plant of tomato. Similar trend was observed in reducing and reducing sugars. There was sharp decline in reducing and non reducing sugar. Estimated reducing and non reducing sugar in healthy plants was 2.05mg /g and 1.48 mg/g respectively whereas it valued 1.16 mg/g and 1.03mg/g respectively in infected plants.

B) Effect on Phenol content

There was increase in phenol content when compared between healthy and infected plants of tomato. Estimated phenol content of healthy and infected plant was 0.042 mg/g and 0.053 mg/g respectively.

C) Effect on Total Protein content

There was sharp increase in protein content in infected plants when compared to healthy one. Total protein content of healthy and infected plants was 0.89 mg/g and 1.024 mg/g respectively.

D) Effect on Total Chlorophyll content

There was sharp decline in total chlorophyll content between healthy and infected plants. It was estimated to be 3.04mg/g in healthy plants whereas it was 2.47 mg/g in infected plant. Similar trend was followed in chlorophyll a and chlorophyll b. In healthy plant, chlorophyll a and chlorophyll b was 1.42 mg/g and 1.62 mg/g respectively whereas in infected plant it valued as 0.93 mg /g and 1.54 mg /g respectively.

Table 1: Comparison of bio-chemical changes in healthy and infected plants

Content (mg/g fresh weight of sample)	Healthy plant	Diseased plant	Percent change over healthy plant
Total Soluble Sugar	3.53	2.19	37.96
Reducing Sugar	2.05	1.16	43.41
Non Reducing	1.48	1.03	30.41
Total soluble protein	0.89	1.024	15.06
Phenol content	0.042	0.053	26.19
Total Chlorophyll content	3.04	2.47	18.75
Chlorophyll a	1.42	0.93	34.50
Chlorophyll b	1.62	1.54	4.94

Discussion

This study is a report on biochemical changes occurred in tomato plants infected with *A. solani*. *Alternaria* produces various toxins such as alternaric acid that causes symptoms on tomato leaves. The first reported example of an *Alternaria* - produced phytotoxin exhibiting the host specificity was from black spot of Japanese pear caused by *A. kikuchiana*.

There was a substantial difference in the chlorophyll level of infected and non infected leaves in the present study. Various plant pathogens are known to produce toxic metabolites, which may destroy the chloroplast resulting into decrease of chlorophyll pigments. The reduction in chlorophyll may be associated to the toxic metabolites produced by pathogen which may destroy the chloroplast or to the inhibition chlorophyll synthesis rather than the degradation of pre-existing pigments.

Reducing sugars were significantly higher in non infected than in infected plants. Decrease in sugar levels may be caused by rapid hydrolysis of sugars during pathogenesis through enzymes secreted by the pathogen. The invading pathogens may utilize the sugar leading to decrease in its content. Gangawane *et al.* (2010) evaluated and compared the biochemical changes in *Alternaria spinaciae* after the application of carbendazim in healthy and sensitive plants. He

observed the amount of non reducing sugar, reducing sugar, total sugar and phenol content is 0.75mg/g, 2.25mg/g, 3mg/g and 1.08mg/g respectively in healthy plants whereas in sensitive plants their amount is 0.48mg/g, 1.14mg/g, 1.25mg/g and 1.44mg/g respectively.

The concentration of total soluble protein increased in leaves treated with the pathogen. During host-pathogen interaction, amino acids act as a substrate for the pathogen or they may have a fungistatic effect through their involvement in metabolic reactions associated with disease resistance. Changes in protein occur when the pathogen penetrates the host cells resulting in disturbances in protein and related metabolisms.

Meena *et al.* (2017) evaluated the biochemical changes in plants of tomato treated with *Alternaria alternata*. He observed that there was significant increase in the total phenol content and total protein content whereas there was sharp decline in the chlorophyll and total soluble sugar as compare to plant that are not treated with *Alternaria alternata*

A gradual increase in total phenol content was noticed with the progress of lesion development. The total phenols were higher in infected leaves than healthy. The post inflectional increase in phenolic contents could be due to their release from glycosidic esters by the enzymatic activity of host or

pathogen, or due to migration of phenols from non-infected tissues.

Conclusion

Based on the present findings, it may be concluded that high level of total phenols, and high protein content of tomato appeared to be the important biochemical constituents, which may impart resistance against infection caused by *A. solani*. Such studies may provide information regarding host-pathogen interaction which can be utilized for resistance breeding for the development of desirable trait by incorporating resistance in promising crop genotypes.

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