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Understanding the Living Process of Whole Organism : A Bio-Chemical Study

Quantitative estimation shows that content of sugar was more in diseased parts than in healthy parts, while the highest amount of starch was observed in healthy part and lowest amount was observed in diseased part. Proteins were found to be higher in diseased parts and lower in healthy parts. Maximum amount of amino acid was observed in healthy parts and minimum amount was observed in infected plant parts. Ascorbic acid and lipids were found to be higher in healthy parts and lower in diseased parts. Phenols were found to be higher in diseased parts and lower in healthy parts.

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Introduction :

Biochemistry, sometimes called biological chemistry, is the study of chemical processes within, and relating to, living organisms. By controlling information flow through biochemical signaling and the flow of chemical energy through metabolism, biochemical processes give rise to the complexity of life. Over the last decades, biochemistry has become so successful at explaining living processes of almost all areas of the life sciences from botany to medicine to agriculture to molecular biology are engaged in biochemical research. Today the main focus of pure biochemistry is in understanding, how biological molecules give rise to the processes that occur within living cells, which in turn relates greatly to the study and understanding of whole organisms.[1]

Much of biochemistry deals with the structures, functions and interactions of biological macromolecules, such as proteins, nucleic acids, carbohydrates and lipids, which provide the structure of cells and perform many of the functions associated with life. The chemistry of the cell also depends on the reactions of smaller molecules and ions. These can be inorganic, for example water and metal ions, or organic, for example the amino acids, which are used for the synthesis of proteins. The mechanisms by which cells harness energy from their environment via chemical reactions are known as metabolism.[2]

The primary metabolites are necessary to sustain the life of the plant and include enzymes, proteins, lipids, carbohydrates and chlorophyll. These are those organic substances, which are synthesized during photo-synthesis and these organic compounds are essential for plant life, growth and development. This aspect of plant biochemistry can be considered as distinct from the production of more complex molecules produced by more diverse pathway.[3]

When plant tissues are affected by a pathogen, deranged (to upset the normal condition or functioning of) metabolic changes are brought about in infected tissues. The pathogen shows responses both by releasing its secretions which compromises auxins, enzymes and toxines to effect nonnalcy in the host cell cytoplasm, including nuclear material. With the development of disease, a complex series of biochemical reactions proceed in an orderly and highly integrated manner. Equilibrium in metabolism is established between host and the parasite in a localized phase of infection.[4] It has been possible to show changes in the metabolism of such transformed cells compared to non-transformed cells. The content of changes varies, according to the nature of the host-pathogen interaction. Enhanced metabolism of the transformed cells is frequently connected with the changes in ultra structures of the cell organelles, changes in carbohydrate contents and chloroplasts during photo-synthesis in plants.[5]

The experimental material for the present investigation consisted of fifteen samples (five samples each healthy, Verticillium Wilt and Solerotinia blight plants), collected from different areas of Rajasthan, India. Infected samples were used after 8 days of infection. T subtract the effect of environment and soil interactions with plant genotype, in case of variability analysis at chemical level, samples were collected and maintained at Department of Biology, University of Rajasthan campus.

Sample Preparation : Crude extracts were prepared by weighing 5 mg approximately of healthy and infected samples and dissolved with 1 ml of double distilled water. Later these solutions were diluted as per the requirement. Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids and terpenoids were carried out for all the extracts by the method described.[6] These tests were carried out in triplicate using various concentrations of samples.

A small portion of crude extracts was dissolved in 5 ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayer's reagent separately. Precipitate or turbidity with the reagents suggests the presence of alkaloids.

A few drops of cone. hydrochloric acid and 1-2

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magnesium turnings were added to I ml of methanolic extracts. The presence of flavonoid was indicated by the development of pink or magenta-red colour.

A small portion of extracts was dissolved in 1ml of chloroform and filtered. To the filtrate on ice, 1 ml of acetic acid was added and then a few drops of cone, sulphuric acid were run down the side of the test tube. The appearance of a pink or pinkish-brown ring /colour indicates the presence of terpenoids. The appearance of blue, bluish-green or a rapid change from pink to blue colours indicates the presence of steroids and a combination of pink and these colours indicates the presence of both steroids and terpenoids.

In both healthy and infected leaves terpenoids, steroids, phenol and favanoid were found to be present, but in infected leaves the quantity of phenol was more (Table 1).

Quantitative analysis of healthy and diseased samples

Phytochemical tests	Healthy plant	Verticillim wilt infected plant	Scleretinia blight infected plant
Alkaloid	+	+	+
Flavanoif	+	+	+
Steroid	+	+	+
Terpinoid	+	+	+
Phenolic Compound	+	+++	+

The following chemical parameters viz, moisture, crude protein, proline, total carbohydrate, starch, soluble sugar, crude fat, crude fiber, phenol, chlorophyll content, neutral detergent fiber (NDF), hemicellulose, cellulose and lignin were studied. The mineral composition of leaf was also studied for phosphorous, magnesium, iron, zinc, potassium and calcium. The moisture content, proline, phenol, total chlorophyll, chlorophyll-a,b and soluble sugar/starch were estimated using the procedures given in/by ICMR (1983), Bates et al. (1973), Bray and Thorpe, (1954), Mahadaven, 1966, Arnon, 1949 and Dubois et a!. (1951), respectively. Fat content, crude fiber, crude protein, total carbohydrate, calcium, potassium, phosphorous, and dietary fiber (Hemicellulose, Cellulose and Lignin) were determined according to AOAC (1995).

Magnesium, iron and zinc were analyzed by using Atomic Absorption Spectrophotometer.[7]

Total Carbohydrate :

Carbohydrates are the important components of storage and structural material in the plants. They are widely prevalent in the form of cellulose of wood and paper, starch, roots, tubers, cane sugar and milk sugars. Chemically they contain the elements of carbon, hydrogen and oxygen, and denoted by the general formula C (H2O)y , where x and y are variable numbers. All carbohydrates are polar and low molecular forms commonly known as sugars. These are freely soluble in water.

Carbohydrate content of the sample was determined by the sum of Nitrogen Free Extract and crude fiber as given below :

Nitrogen Free Extract (%) = 100 - (% Crude Protein + % Ether Extract + % Crude Fiber + % Total Ash)

Total Carbohydrate = % Nitrogen Free Extract + % Crude Fibre

In the present studies, total carbohydrate percentage was obtained as follows:

For healthy plant = 6.3%

For Verticillium wilt infected plant = 6.7%

For Sclerotinia blight infected plant = 6.8%

Assessment & Soluble Sugar :

The Soluble Sugar was estimated by Anthrone (1951) method. 0.1 to 0.5 g of the sample was homogenized in hot 80% ethanol. It was centrifuged and supernatant was taken for the estimation of soluble sugars. Then, 0.1 or 0.2 ml of the supernatant was pipetted out and volume was made 1 ml with water. 1 ml of sample was taken and 4 ml of Anthrone's reagent was added to each tube. It was heated for 8 minutes in a boiling water bath. It was then cooled rapidly and the intensity of green to dark green colour was measured at 620 rim. The Soluble Sugar content in the sample was calculated using the standard graph.

In the present studies, total soluble sugar percentage was obtained as follows:

For healthy plant = 2.7%

For Verticillium wilt infected plant = 2.9%

For Sclerotinia blight infected plant = 3.1%

Assessment of Starch :

The starch was estimated by Anthrone's method (1951). Starch is a substance that plants use to store energy. It is the product of photo-synthesis and can be stored for later use in seeds, tubers and roots. Chemically, starch is a polysaccharide comprised of glucose molecules linked in long chains.

0.1 to 0.5 gm of the sample was homogenized in hot 80% ethanol to remove sugars. It was centrifuged and the residue was retained. The residue was washed repeatedly with hot 80% ethanol and the residue was dried well over water bath. To the residue 5.0 ml of water and 6.5 ml of 52% perchloric acid was added. It was extracted at 0°C for 20 minutes. It was centrifuged and supematant was saved. The extraction was repeated using fresh Perchioric acid. It was then centrifuged and the supernatants were pooled and volume was made to 100 ml. From the supematant, 0.1 or 0.2 ml of was pipetted out and volume was made up to 1 ml with water. Subsequently, I ml of sample was taken and 4 ml of Anthrone's reagent was added to each tube. It was heated for 8 minutes in a boiling water bath. It was cooled rapidly and the intensity of green to dark green colour was measured at 630 nm.

Conclusion :

In the present studies, quantitative estimation shows that content of sugar was more in diseased parts than in healthy parts, while the highest amount of starch was observed in healthy part and lowest amount was observed in diseased part. Proteins were found to be higher in diseased parts and lower in healthy parts. Maximum amount of amino acid was observed in healthy parts and minimum amount was observed in infected plant parts. Ascorbic acid and lipids were found to be higher in healthy parts and lower in diseased parts. Phenols were found to be higher in diseased parts and lower in healthy parts.

References :

(1) *Hugenholtz et al., 2013.* ***(2)*** *Woese et al., 2013.* ***(3)*** *Hugenholtz, 2002.* ***(4)*** *Sharma, 2004.* ***(5)*** *Mullet et al., 1990.* ***(6)*** *Harbome (1998) and Sazada et al. (2009).* ***(7)*** *Model: GBC - 932, AA.*