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Biodeterioration of active principles of *Catharanthus roseus* by *Colletotrichum gloeosporoides*

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Abstract

Majority of the members of Coelomycetes are diseases causing mostly associated with leaf spots. Leaf spot diseases are the expressions of abnormal metabolic changes due to loss of photosynthetic area. Such symptoms are produced as a result of physiological disorders in the host. These disorders may be due to nutritional imbalance or due to living organisms, usually active in localized areas of the host. Leaf spot diseases are known to cause severe damage to the plants of great economic and ornamental value. Cultivated plants are usually more susceptible to such diseases. These diseases, which deprive the plants of the aesthetic value and cause a decrease in photosynthetic area, require greater probe and attention. A general survey undertaken by the research team of TMBU under AICOPTAX Program, MoEF, New Delhi showed greater frequency of Coelomycetous fungi. Particularly *Colletotrichum gloeosporoides* which have been selected in the present research work for the study in detail. A general pattern of decrease of sugars, biomass and alkaloid contents were observed after pathological studies of *C. roseus*. The percentage decrease in Total Sugar was found 30.36%, Reducing Sugar 28.57%, Non-Reducing Sugar 30.61%, biomass (7%) Alkaloids 21% under pathogenesis.

Key words: Coelomycetes, Biodeterioration, Medicinal plants.

Introduction

The Coelomycetes are predominantly leaf spotting fungi though many of them grow on twigs, fruits and other parts of the plants causing different symptoms like blight, rot, cankers etc. The pycnidia are with or without the ostiole and the acervuli are saucer shaped structure which are the characteristics of this group. The present work deals with taxonomical and pathological studies of Coelomycetous fungi associated with medicinal plants. The main object of the present investigation was to explore the diverse localities of Eastern Bihar to collect different nature of genera of Coelomycetous fungi associated with medicinal and the plants of other economic importance. As early as in 1972 Vidyashekharan and Kandaswamy [1] showed severe reduction in both starch and sugar contents of infected tissue of *Phaseolus aureus*. The observation [2] supported this findings the facultatine parasite such as *Cercospora* was found to deplete starch and sugar contents in groundnut and banana leaves [3], also found a quick decline in sugar content of banana due to *Cercospora* infection. The simple sugars are preferably assimilated by pathogens as carbon source which determines the quality of the fruits and leaves [4-8]. The impact of coelomycetous fungi on medicinal plants as a whole or on deterioration of carbohydrates and active principles has been studied by several earlier workers [9-16], moreover, it still requires further studies to generate scientific information by plant wise in relation to severity of fungal diseases. Keeping these in view changes in leaf photosynthates, biomass & alkaloids of *Catharanthus roseus* due to fungal disease caused by *C. gloeosporoides* have been studied.

Materials and Methods

Estimation of Total Sugar: To 200 mg of each sample, 25 ml hot 80% ethanol was added and stirred thoroughly. After 5 minutes, it was centrifuged and supernatant was decanted. Extraction was repeated by adding 30ml of hot 80% ethanol. The extracts were mixed and ethanol was evaporated to dryness in an evaporating disc. The residue left at the bottom was dissolved in 5ml of glass-distilled water and centrifuged again. To 2ml of the supernatant 0.14 ml of 80% aqueous phenol was added and then with fast flowing pipette 5 ml of conc. H_2SO_4 was mixed and shaken gently. The tubes were allowed to stand for ten minutes and then placed in a water bath at 25°C for 10-20 minutes. The optical density was recorded against the blank at 490nm. The percent amount of the total sugar was determined by comparing the readings with that of standard curve of glucose [17].

Estimation of Reducing Sugar: 300mg of each sample was finely crushed and blended with 1.5ml of glass distilled water in a glass homogeniser. To this 0.2ml of 0.3N Barium hydroxide solution was added followed by 0.2ml of 5% $ZnSO_4$ solution and was thoroughly mixed. The total volume was centrifuged. To one ml of the supernatant, one ml of alkaline copper reagent (Prepared by dissolving 4 gm $CuSO_4$,

$5H_2O$; 24 gm Anhydrous Na_2CO_3 and 16 gm Na-K tartarate in 1 liter of water) was added. To this one ml of Arsenomolybdate reagent was added and left to stand for few minutes till the effervescence ceased. The blue colour was diluted with glass distilled water up to 10ml and was read at 510 nm. The optical density was compared with that of the standard fructose solution [18].

Estimation of Non-reducing Sugar: The amount of non-reducing sugar was calculated by subtracting the value of reducing sugar from total sugar. It is because the total sugar constitutes equally reducing and non-reducing sugars.

Loss in Biomass: The test plant (*Catharanthus roseus*) was inoculated artificially by the test fungus (*Colletotrichum gloeosporoides*). The plant after artificial infection was left for 15 days for the development of disease symptom under an aerated poly bag. After 15 days symptoms was appeared on the leaf surface. The healthy and diseased leaves were detached separately. weight. was taken after proper washing and kept into oven at 60-80^oC for 48 hours. The biomass was calculated by the following method: -
For healthy leaves: Wt. of healthy fresh leaves – Wt. of oven dried leaves.
For diseased leaves: Wt. of diseased leaves – Wt. of oven dried leaves.

Changes in Total Alkaloid: 20gm of powdered sample was soaked in 28% ammonium hydroxide solution and little dried-up. Subsequently the sample was soxhlated with a mixture of chloroform & ethanol (3: 1 v/v) for 8 hours. 100ml of solvent was extracted and vigorously shaken with 25ml of N/2 H_2SO_4 and the acid extract was collected. The process was repeated thrice for the complete extraction of alkaloids. The combined acid extract was made alkaline with dilute NH_4OH. The alkaloids were extracted from alkaline extract with 20 and 15ml of chloroform. The chloroform extract was distilled on water bath until only few ml was left. The left solvent was completely dried up and left residue was weighed on monopan balance to calculate the total crude alkaloids [19].

Results and Discussion

Changes in sugar: Carbohydrates or sugars are the chief photosynthetic products of the plants. The utilization of any particular sugar by fungi depends on its configuration as well as on the potential endowment of specific fungi. Fungi satisfy their carbon requirements from various carbohydrates present in host tissues by (i) Breaking the complex sugars to simpler utilizable form and by (ii) Enhancing the respiratory rate in diseased tissues. Di- and Polysaccharides are hydrolyzed by various hydrolyzing enzymes and are converted into hexose sugars which are preferably used by various pathogenic fungi mainly for two purposes i.e. to augment the required amount of energy for various metabolic process and for structural frame work of cell however, the rate of utilization varies with the variation of nature and type of fungi.

Loss In Biomass: All living plant cells requires an abundance of water and an adequate amount of organic and inorganic nutrients in order to live and to carry out their physiological function. Plant absorbs water and inorganic (mineral) nutrients from the soil through their root system. The minerals and part of the water are utilized by the leaf and other cells for the synthesis of the various plant substances, but most of the water evaporates. On the other hand, nearly all organic nutrients of plants are produced in the leaf cells, following photosynthesis and are located downward and distributed to all the living plant cells. When a pathogen interferes with the upward movement of inorganic nutrients and water with the downward movement of organic substances, diseased conditions results in the parts of the plant denied these materials. The diseased parts in, turn, will be unable to carry out their own functions and deny the rest of the parts, their services or their products. For example if water movement to the leaves is inhibited the leaves cannot function properly, photosynthesis is reduced or stopped and few or no nutrients are available to move to the roots, which in turn become starved and diseased and may die or resulting in the loss of biomass [20-22]. In the present study two months old healthy and diseased leaves at *C. roseus* was taken for the determination of loss in biomass and the results are depicted in table 1.

Changes in Alkaloid Contents: The term Alkaloid has been proposed by pharmacists as basic nitrogen containing compounds from the plants and other natural resources in which at least one nitrogen atom forms a part of the cyclic system. The above stated definition is not fully satisfactory because a number of synthetic compounds satisfy all the above criteria of alkaloid except for the facts that they are not derived from biological resources. Thus in the modern chemistry they are defined as heterocyclic nitrogenous compounds. The further work carried out [23-29] suggested that some proteins, amino acids, nicotinic acid and anthranilic acid may act as precursor for the biosynthesis of alkaloid. In general alkaloids have been isolated and characterized from almost all the parts of the plants; however, their concentration is more in those parts where the metabolic activity is maximum. The curative properties of alkaloids against various diseases have led their wide range use in medicine preparation at a large scale. 30 and 31 listed number of plant alkaloid viz. Atropine, Caffeine, Cocaine,

Codeine, Emetine, Morphine, Ergometrine, Serpentine and Quinine which are used to cure different ailments of human being. Previously it was reported [32] that Reserpine obtained from *Rauvolfia serpentina* have a strong hypotensive property, Vinblastine and Vincristin isolated from *C. roseus* as well as ergine, ergometrine, ergoline and isergine isolated from *Argeria* sp. are antidiabetic and hypoglyceamic [33-36].

Table 1: Changes in Sugar contents, Biomass and Alkaloids of *C. roseus* by *C .gloeosporoides*.

		Healthy	Diseased
Total sugar (TS)mg/g	TS	5.6	3.9
	% loss	-	30.96
Reducing sugar (RS)mg/g	RS	0.7	0.5
	% loss	-	28.57
Non reducing sugar (NRS)mg/g	NRS	4.9	3.4
	% loss	-	30.61
Biomass (B)mg/g	B	8.7	-
	% loss	8.1	7
Total Alkaloid (TA)	TA	0.81	-
	% loss	0.63	21

The data in table 1 clearly reveals that there was a general pattern of decrease of all kinds of sugars. 5.6mg/g total sugar, 0.7mg.g reducing sugar and 4.9mg/g non-reducing sugar in healthy leaves whereas only 3.9mg/g, 0.5mg/g and 3.4mg/g were estimated from diseased leaves samples respectively. The biomass of healthy and diseased leaves was recorded 8.7g/50g, and 8.1g/50g respectively. The amount of total alkaloid in healthy leaves was found 0.81 mg/g and 0.63 mg/g in diseased one. The percentage decrease in total sugar, biomass and alkaloids was found to the tune of 30.96%, 7% and 21% respectively under pathogenesis. The growth of a plant or its particular organ is an important endogenous process which results in variation in the concentration of plant metabolites. The percent decrease in diseased leaves might be due to the utilization of organic and inorganic components by the test fungus (*C. gloeosporoides*).

Conclusions

A general trend of decrease in sugar content of *C. roseus* was observed. Total sugar (TS) decreased by 30.36%, reducing sugar (RS) by 28.57% and non-reducing sugar by 30.61% respectively. The biomass was found to decrease up to 7% after the infection by *C. gloeosporoides*. The total alkaloid content in healthy leaves of *C. roseus* was 0.81 mg/g fresh leaves, however in diseased leaves it was decreased by 21%.

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