Research Paper

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Estimation of Nucleic acid content and phenol content in *Syzygium* cumini (L.) Skeels affected by *Cassytha filiformis* (L).

BOTANY

KEYWORDS : Nucleic acid, phenol, healthy leaves, affected leaves.

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ABSTRACT

Nucleic acid and phenol content were estimated in healthy and affected leaves of S. cumini. It was economically, medicinally important plant, estimation of nucleic acid content (DNA&RNA) in affected leaves showed higher level than healthy leaves (range mg/g fresh/wt), phenol content was showed higher level in affected leaves than healthy leaves.

INTRODUCTION

Syzygium cumini is native to South Asia; this tree belongs to the family Myrtaceae. All parts of these plants are medicinally useful and it has a long tradition in alternative medicines. The genus comprises 1100 species, extends from Africa to Madagascar through Southern Asia east through the pacific. Botanically it is *S. cumini,* commonly known as jamun, is a medicinal plant and utilizable species. Common names are java plum, Blank plum, Jambul and Indian Black berry (Nadkarni, 1954). The wood is water resistant because of this it is used in railway sleepers and to install motors in wells. It is sometimes used to make cheap furniture and village dwellings though it is relatively hard to work on.

S. cumini is a fast grown medicinally important plant with such abundant utility suffers from **higher plant parasite** (***Cassytha filiformis***) all over the world, resulting wilting, reduction in plant size, loss of yield or low yield, lower quality, physiological changes in the host plant after *Cassytha filiformis* attack.

Host - Parasitic relationships

It is often stated that a good parasite does not kill its host. Variation in the degree of pathogenicity exhibited by various parasitic plants is great; from those they exert little impact on their hosts to those that dramatically affect the host physiology.

MATERIAL AND METHODS

Collection of plant material for analysis

The leaves of *S. cumini* were collected from healthy and affected branches of parasitized tree at different seasons of summer (April-June) (32-40^{0}C), winter (December-March) (20-25^{0}C) and Monsoon (July-September) (15-20^{0}C) in the Nelapattu, Forest area, Nellore dist. The leaves were located in external, middle part of the crown with same position towards sun light direction.

About 100 leaves (including) petioles were picked and placed in to tightly sealed nylon bags and immediately transferred to the laboratory with minimum delay. Petiole and viens were removed they were washed thoroughly with distilled water and used immediately for extraction for bio-chemical and enzymatic analysis of host parasite complex carried out by using standard methodology.

Macroscopic study of host leaves

The leaves were measuring about 10 to 15 cm long and 4 to 6 cm wide. These are entire, ovate, oblong, sometimes lanceolate and also acuminate, coraceous, tough and Smooth with shine above. The fragrant flowers of jamun are small, nearly 5mm in diameter; these are arranged in terminal trichotomous panicles greenish white in colour.

Nucleic acid content and phenol content variation of the Host – Parasite complex

Host-Parasite complex was analyzed using infected plant leaf extract / filtrate extract for various bio-chemical constituents like Nucleic acid content and phenol content using standard procedures.

1. Nucleic acids

Nucleic acids (DNA and RNA) were extracted and estimated according to the method of Nieman and Poulson (1963) from both healthy and affected leaf tissues at three seasons of disease development.

Extraction: About 250 mg of freshly harvested leaf material was extracted with cold methanol. Centrifuged and the residue repeatedly washed with cold methanol to remove pigments and alcohol soluble phosphorous. To the residue, 5% ice cold trichloro acetic acid (TCA) was added, centrifuged and the resulting residue was washed twice with 90% ethanol and ether (ethanol ether (2:1) v/v). For each extraction, the solvent was allowed to boil for 20 seconds. The residue was carefully dried, powdered and used for further extraction of RNA and DNA.

Estimation of RNA

The dried residue was suspended in 5 ml of 0.3 N sodium hydroxide and held at 30^{0}C for 18 hours. The sediment was centrifuged and washed with 5 ml of 0.3 N sodium hydroxide, Both the wash and extract were combined, made to 10 ml with 0.3N NaOH, acidified to P^H 1.0 with 15% PCA, held at 4^{0}C for 40 min and then centrifuged. The supernatant obtained contained the RNA fraction.

The DNA (protein) sediment was resuspended with 2ml water followed by the addition of 1N perchloric acid (PCA) the suspension, held at 4^{0}C for 20 min was centrifuged and the supernatant added to the RNA fraction before bringing the final RNA fractions up to ***20 ml with distilled water.***

Estimation of DNA

The DNA present in the sediment was suspended in 3 ml of 0.5 N PCA, ***homed heated at 70^{0}C for 15 min, centrifuged at 4000 rpm at 2°C and the wash added to*** the DNA fraction. The final DNA extract was made up to 5 ml with 0.5 N PCA ***and the DNA extract was measured using 0.5 N PCA as the blank. O, D val-***

ues were measured at 260 nm using a Schimadzu spectrophotometer and using sperm DNA and yeast RNA respectively as standards.

2. Total phenols

The quantitative determination of total phenols was estimated according to the method of Swain and Hills (1959) where about one gram of freshly harvested ***leaf samples were boiled in 5 ml of 2 N HCl on a water bath for 20 min and filtered. The residue obtained was ground in 5 ml of 2 N HC1, filtered; the filtrates were pooled and then centrifuged at 3000 rpm for 15 minutes. The supernatant was extracted twice with 20 ml of diethyl ether. Both diethyl ether extracts were pooled, evaporated and the residue was dissolved in 3 ml of 95% ethanol and diluted to 7 ml with distilled water. To the mixture, 0.5 ml folin-phenol reagent was added, shaken thoroughly and held for three min. Before adding 1.0 ml of saturated sodium carbonate solution and making up to 10 ml with distilled water.*** The blue colour intensity developed after one hour was measured at 725 nm in a schimadzu UV -240 spectrophotometer using coumarin as the standard solution.

RESULTS AND DISCUSSION

BIOCHEMICAL ANALYSIS OF HOST – PARASITE COMPLEX

Quantitative changes in Nucleic acid content and phenol content were determined in both healthy and affected S. cumini leaves at each of the three seasons.

All the results were computed on gm fresh weight basis and changes in various constituents due to infection expressed as percent increase (marked by '+' sign) or decrease (marked by '–'sign) over healthy plants at different seasons of infection / disease development.

Table-1: Nucleic acid content (mg/ g fr.wt)* in healthy and *C. filiformis* affected *S. cumini* leaves at different seasons of infection

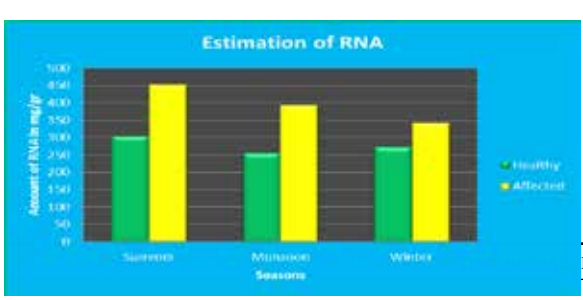
Seasons	RNA			DNA		
	S. cumini			*S. cumini*		
	Healthy	Affected	POC	Healthy	Affected	POC
Summer	303.27 ±0.02	452.23 ±0.01	49.15	195.36 ±0.01	338.13 ±0.02	73.08
Monsoon	254.62 ±0.02	394.54 ±0.02	54.95	143.633 ±0.25	214.25 ±0.03	49.16
Winter	272.54 ±0.02	343.26 ±0.02	25.95	183.633 ±0.01	229.18 ±0.02	24.83

*Average values of triplicate samples
POC – Percent Over Control
± Standard deviation

Table-2: Estimation of phenol content (mg/ g fr.wt) in healthy and *C. filiformis* affected *S. cumini* leaves at different seasons of infection

Seasons	*S. cumini*		
	Healthy	Affected	POC
Summer	5.62 ± 0.02	6.34 ± 0.02	12.81
Monsoon	4.363 ± 0.015	5.23 ± 0.01	19.93
Winter	4.856 ± 0.03	5.75 ± 0.01	18.46

*Average values of triplicate samples
POC – Percent Over Control
± Standard deviation



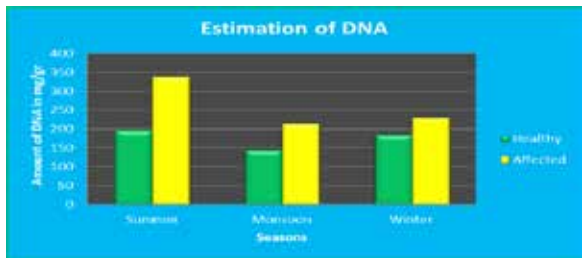


Fig-1: Bar diagrams showing the changes Nucleic acid content (mg/ g fr.wt) of healthy and affected *S. cumini* leaves

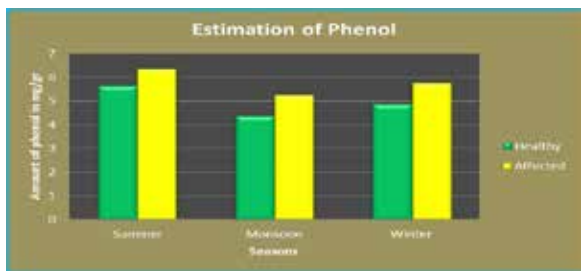


Fig-2: Bar diagrams showing the changes phenol content (mg/ g fr.wt) of healthy and affected *S. cumini* leaves

In the present study, Nucleic acid content and phenols were observed in infected leaves of the host *S.cumini* by *C.filiformis* was compared with healthy host leaves. Significant variations were observed in the two parameters.

Nucleic acids: In the affected *S. cumini* plants both RNA and DNA contents were higher. The two nucleic acids (RNA and DNA) quantity increased with the age of the plant in both healthy and affected leaves of S. *cumini* (Table-1 and Fig-1).

RNA: It was high in affected *S. cumini* leaves by 49.15%, 54.95%, and 25.95% at different season's summer, monsoon and winter respectively when compared to the corresponding healthy samples. The RNA content was greater at monsoon, summer decreased at winter season.

DNA: It was more in affected plants by 73.08%, 49.16%, and 24.83% at different seasons respectively as compared to the corresponding healthy samples. The DNA content was greater at summer, monsoon decreased at winter seasons.

The increased nucleic acid content may be due to the contribution by the parasite accumulated in the tissues or enhanced activation of host nucleic acid synthesis as a result of infection or both.

Nucleic acid contents- RNA in healthy leaves decreased compared to affected leaves in all the three seasons, also DNA contents decreased in the healthy leaves compared to affected leaves this is in live with the changes as observed by Suryaprakash *et al.* (1967) in their studies in the leaves of *Loranthus* affected host plants. Increase of the two nucleic acids in the affected plants might be either due to enhanced activation of host nucleic acid synthesis as a result of infection or disturbance caused by the parasite/ its metabolites during development. A study on the synthesis of nucleic acids in healthy and affected plants may yield useful information.

Total phenols: Total phenols content was higher in affected leaves than that of healthy leaves of *S.cumini* (Table-2 and Fig-2). In the affected plants the content was greater by 12.1%, 19.9% and 18.4% at different seasons when compared to healthy plants. The phenolic content was increased at monsoon, winter and

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then decreased in summer.

The total phenols were higher in affected leaves than that of healthy leaves of *S. cumini*. This suggests an increased synthesis of phenols not only with aging of plants but also their further stimulation as a result of infection the most striking effect of infection was on phenol compounds, the effect was both qualitative and quantitative phenol compound were detected in healthy leaves.

Analysis of the phenol composition in the healthy leaves of *S. cumini* and affected (*C. filiformis*) leaves showed decreased contents in healthy compared to affected leaves. The increase in total phenol and other constitutive phenols observed in the study have been reported by others using different plant-pathogen interaction (Chattopadhayay and Bera, 1980; Khan *et al*., 2001).

CONCLUSION

From an overall consideration of the results of the affected plants it may be concluded that *C. filiformis* interferes with various biochemical and enzyme biosynthesis mechanisms of the host plant for the benefit of the parasite growth and reproduction, it was clear that the parasite *C. filiformis* continuously was absorbing the host plant's nutrients and metabolites namely nucleic acid content (DNA & RNA) and Phenols as a results reducing the yield potentials of the host plant. Any adverse effect by this parasite may lead to the destruction of any or all of these medicinally and economically important plants, Hence, the control of this parasite are the need of the hour.

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