Quantitative Estimation of Some Metabolites in *Syzygium cumini* (L.) Skeels. Affected With *Cassytha filiformis* L.

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Abstract- The seasonal variation of nitrogen and total lipids content has been investigated *S.cumini* leaves affected with *Cassytha filiformis. Syzygium cumini* are the medicinally important plant, comparative account of nitrogen content of healthy leaves showed high level (range mg/g dry wt) than affected leaves (range mg/g dry/wt.). The healthy leaves are of showed high level of lipids content (range mg/g/dry wt.) than affected leaves.

Index Terms: *Syzygium cumini*, *Cassytha filiformis*, Nitrogen, Lipids, Healthy plant, affected plant.

Introduction

Syzygium cumini is an evergreen plant which is originally being grown in forest lands of Indonesia and India. This tree belongs to the family Myrtaceae. All parts of these plants are medicinally useful and it has a long tradition in alternative medicines. In these days herbal medicines are more popular than modern medicine because of their effectiveness, easy availability, low cost and for being comparatively devoid of side effects.

S. cumini is native to South Asia, 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). It can reach heights of up to 30m and can live more than 100 years. At the base of the tree the bark is rough and dark grey, becoming lighter grey and smoother higher up. 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 tree starts flowering from March to April. The flowers are fragrant and small, about 5mm in diameter. The fruits develop by May or June and resemble large berries. The fruit is oblong, ovoid, starts green and turns pink to shining crimson black as it matures. A variant of the tree produces white colored fruit. The fruit has a combination of sweet, mildly sour and astringent flavor and tends to colour the tongue purple.

Economic importance

The fruit tree has great economic importance (Mir *et al.,* 2009). Almost all parts of the tree are used for various purposes. *S. cumini* fruits are a very rich source of antioxidants and have numerous healthy benefits. Ripe fruits are very juicy, almost odorless, with a pleasant, slightly bitter, astringent taste, the fruit pulp is used to make jam, jellies, juice, vinegar and puddings. Fruits are also used to make wine in vast an antitoxins in the Philippines. Fruits are used as antioxidant, (Brito *et al*., 2007) anticancer (LiL *et al.*, 2009) antihyperlipidemic (Rekha, 2010).

S. cumini showed an antimicrobial effect against enteric bacteria antibacterial activity. Different types of fruits have been used to prepare jams, jellies, syrups, and ice creams since ages (Chaudhary *et al.*, 2012). The fruit is used removes bad smell from mouth, stomachic, astringent, diuretic and antidiabetic (Nadkarni 1976). Treatment of chronic diarrhea and other enteric disorders (Veigas *et al.*, 2007). Juice of the ripe fruit is an agreeable stomachic, carminative and diuretic (Kiritikar *et al.*, 1987). It is also useful in spleen enlargement and an efficient astringent in chronic diarrhea, Fruit juice is good quality and excellent for sherbet, syrup and squash an Indian drink, In unani medicine various parts of jambolan act as liver tonic, enrich blood, strengthen teeth, and gums, and form good lotion for removing ring worm infection of the head (Sagrawat, *et al.,* 2006). Fruits are used to make wine, which is produced in vast quantities in the Philippines.

The leaves and bark are used for controlling blood pressure and gingivitis. Wine and vinegar are also made from the fruit. It has a high source of vitamin A and vitamin C (Luximon-Rammal *et al.,* 2010) the ash of the leaves is used for strengthening the teeth and gums, leaves may be used as fodder**.**

Parasitic flowering Plants on *S. cumini* tree

Parasitic plants derive some or all of their energy from other plants, and about 4100 species of parasitic flowering plants in 270 genera are known (Nickrent and Musselman, 2010). There are four major types of parasitic plants based on whether they can photosynthesize (hemiparasite)or not (holoparasite); and whether the parasite is attached to the host plant above ground or below ground (Heide-Jorgensen, 2008).

Cassytha filiformis

The Genus *Cassytha* (Lauraceae) is a perennial hemi parasitic vine that attaches to the stems of host plants (Heide-Jorgensen, 2008). This genus is the only parasitic taxon in the Lauraceae, and comprises 23 species in the Old and New World tropics and subtropics (Weber, 2007). Mature plants of *Cassytha* have twining stems without roots or developed leaves (Weber, 1981). Soon after germination, *Cassytha* plants develop radicles that grow into the soil, and produce a few short lateral roots using nutrients stored in the cotyledons. Once the first haustorium forms, the radicles disappear and the plant becomes independent of soil contact (Heide-Jorgensen, 2008). Host-specificity of parasitic plants has been studied in several fields, including ecology (Cuevas- Reyes *et al.*, 2011), evolutionary biology (Thorogood *et al.*, 2009) and physiology (Fernández-Aparicio *et al.*, 2011). However, host - specificity has been poorly investigated for the *C. filiformis* in the present studies, the host plants of *C. filiformis* were surveyed in Chittoor and Nellore dist of A.P and discussed relationships of host specificity.

Cassytha is a **laurel Dodder** and *Cuscuta* is a **Dodder** is on woody plants. The wide range of hosts attacked by Dodders is reviewed in (Dawson *et al.*, 1994) simultaneous attached of a single parasite to several diverse hosts. The host range of *Cassytha* is broad. (*C. filiformis* is pantropical and is also abundant in southern florida) species are superficially similar, and so a new introduced species could easily be over looker.

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 (e.g. *Epifagus* on *Fagus*) to those that dramatically affect the host physiology and fecundity (e.g. *Striga* and *Orobanche* on various crop plants). Pathogenicity depends upon many factors, such as the biomass ratio of parasite to host,

the number of parasites attached to an individual host plant, the length of time required for the parasite to complete its life cycle, and possibly the degree of coevolutionary "tuning" that has occurred over time between the two species. Complicating factors include examples such as *Striga* on cereals.

Parasitism

Parasitism is the interspecific interaction (Estabrook EM and Yoder JI; 1998). Where one of the species (Parasite) depends the other species (Host) for food and shelter, host gets damaged due to parasite where as parasite get benefitted. Parasites are host – specific and parasite and its host tend to co – evolve. Co – evolution means that if the host evolves a special mechanism to reject or resist the parasite, the parasite has to evolve the mechanisms to counteract and neutralize them, in order to be successful with the same host species. Parasites have complex life cycle and generally have one or two intermediate host. Intermediate hosts act as vectors which facilitates parasitisation of host (Michael Conrad 1983).

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 veins were removed they were washed throughly 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.

Biochemical analysis of the Host – Parasite complex

Host-Parasite complex was analyzed using infected plant leaf extract / filtrate extract for various bio-chemical constituents like nitrogen,and lipids, using standard procedures.

1. Nitrogen fractions

Total nitrogen and soluble protein nitrogen fractions were estimated in leaves of healthy and infected *S. cumini* plant.

a) Total Nitrogen

It was estimated according to the method of Markham (1942). One gr of fresh material was taken in a 25 ml micro Kjeldahl flask to which about 100 mg of catalyst (1gr $CuSO_4$ + 5g K_2SO_4 +1g SeO_2) was added to aid digestion. Three ml of fuming concentrated analytical grade sulphuric acid and 1ml of hydrogen peroxide were also added and the sample was allowed to digest on a hot plate until a clear colourless solution was obtained. The final volume of the solution was made up to 25 ml with distilled water. 5ml aliquots of the digest were transferred to a distillation unit and 10ml of 40% sodium hydroxide was added just prior to distilling for 20 minutes. The ammonia liberated was absorbed in to a boric acid –indicator mixture in a volumetric flask. The completion of distillation was recognized by the change in p^H of the indicator solution in the receiver from pink to green. After complete distillation, the indicator solution was titrated against 1N Hydrochloric acid until the pink colour reappeared. The amount of nitrogen present in the sample was calculated as 1m of 1N HCL=0.14 mg of nitrogen and the results were expressed as mg per gram fresh weight.

Boric acid indicator mixture: 10g of boric acid and 200ml of absolute alcohol were mixed and added to the indicator solution consisting of 0.033mg bromocresol green and 0.666 mg of methyl red in 100 ml of absolute alcohol. This indicator mixture was made up to 1 litre with distilled water and the pH was adjusted to 5.0.

b) Protein Nitrogen

Protein nitrogen was estimated by the method of Thimann and Loss (1957) as follows. About one gram of fresh material was macerated with 10 ml of 10% TCA at 4°C and centrifuged at 2000xg for 30 minutes. After decanting the supernatant, the precipitate was washed with 5 ml of 5% TCA. Again 5 ml of 5% TCA was added to

the washed precipitate and the mixture was incubated for 30 min at 80°C to remove nucleic acids. The precipitate obtained after centrifugation was analyzed by the micro kjeldahl method as mentioned above for total nitrogen.

c) Soluble Nitrogen

Soluble nitrogen was estimated by subtracting protein nitrogen from total nitrogen. The results were expressed as mg nitrogen per gram fresh weight of the sample.

2. Lipid fractions

a) Total Lipids

Total lipids were extracted from leaf samples of both healthy and affected by the method of Hoppe and Heitefuss (1974).One gram of leaf sample was homogenized in 22 ml of boiling solvent mixture consisting of 6 ml chloroform, 12 ml methanol and 4 ml water (1:2:0.8 v/v/v), filtered and reextracted with 14 ml methanol in a mortar. The residue was washed with 20 ml chloroform. All the extracts were combined in a separating funnel and 18 ml of water was added to make the ratio of chloroform-methanol- water to 2:2:1:8 (v/v/v).After removing the lipid layer, the water layer was extracted thrice with 20 ml chloroform. These layers were evaporated to dryness at 43-45^{0}C in a rotary evaporator. Two ml of Benzene was added to the residue and evaporated under nitrogen. The dried lipid residue was quantitatively dissolved in chloroform in to a previously weighed bottle and evaporated to dryness under nitrogen. The bottle was kept in a vacuum desicator over KOH under reduced pressure for several hours and again weighed. The weight of total lipids present in one gram leaf samples was calculated by taking the difference in the above weights and recorded as mg/g fresh weight. The lipid residues were dissolved in chloroform in 5 to 10 ml volumetric flasks and stored in a deep freeze for further studies.

b) Total Glycolipids

Estimation of total glycolipids was done by the Phenol-sulphuric acid method of Dubois *et al., as* described by Roughan and Batt (1968). An aliquot of the lipid extract was placed in a test tube, evaporated to dryness and 0.9 ml Methanol and 0.1 ml 5 N NaOH were added. A marble was placed over the tube and the mixture heated for an hour at 45-50°C in a water bath. The hydrolysate was evaporated to dryness under compressed air, 1 ml of 2N H_2SO_4 was

added to the residue. A marble was placed on the tube and it was again hydrolysed for $2^1/_2$ hours on a boiling water bath. After hydrolysis, 1 ml chloroform was added to partition pigments. To an aliquot from the aqueous phase, 0.5 ml water and 0.5 ml 4% phenol were added and shaken well. Four ml of concentrated sulphuric acid was added and the contents of the tubes were thoroughly mixed and allowed to stand at room temperature for 15 min before reading the colour intensity at 480 nm in a Schimadzu spectronic spectrophotometer. Galactose standards containing 10 to 20 μg / tube were run simultaneously each time and the concentration of glycol lipids was expressed as mg/gm fresh weight of the material.

c) Total Phospholipids

To determine the phosphorus content in phospho lipids, Bartlett's (1959) procedure with a slight modification was followed. Lipid samples containing 1 to 10 µg of phosphorus were placed in a test tube marked in 10 ml and the organic solvent was removed by passing compressed air. 1 ml of 60 % perchloric acid was added to each tube and the samples were digested at 170-180^{0}C in a heating block until the samples were clear. After digestion, 4.5 ml of 0.44% ammonium molybdate (4.4 g of ammonium molybdate added to 14 ml concentrated sulphuric acid made to one litre) followed by 0.2 ml of 1-amino, 2-naphthol, 4-sulphonic acid (0.5 g 1-amino, 2-naphthol, 4-sulphonic acid, 6 g sodium sulphite, 30 g sodium bisulphate dissolved in 250 ml distilled water) were added to each test tube. The contents were mixed thoroughly, heated in a boiling water bath for 10 minutes, cooled and the volume was adjusted to 10 ml with glass distilled water. Colour intensity was measured in a Schimadzu spintronic spectrophotometer at 660 nm wave length. Aliquots of KH_2PO_4 solution containing 2µg of phosphorus/ml were used as a standard and simultaneous blanks were maintained. Total lipid content was calculated by multiplying the lipid phosphorus by 25 and expressing it as µ moles of phosphorus per gram fresh weight of the material.

RESULTS AND DISCUSSION

BIOCHEMICAL ANALYSIS OF HOST – PARASITE COMPLEX

Quantitative changes in nitrogen, Total lipids 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: Estimation of nitrogen fractions of healthy and *C. filiformis* affected *S. cumini* leaves at different seasons of infection.

Seasons	Total Nitrogen (mg/g)			Protein Nitrogen (mg/g)			Soluble Nitrogen (mg/g)		
	S. cumini			*S. cumini*			*S. cumini*		
	Healthy	Affected	POC	Healthy	Affected	POC	Healthy	Affected	POC
Summer	22.86 ±0.01	18.44 ±0.01	-19.32	19.96 ±0.02	16.83 ±0.01	-15.68	2.81 ±0.01	2.6 ±0.02	-7.47
Monsoon	20.9 ±0.01	14.06 ±0.02	-32.72	19.42 ±0.015	13.82 ±0.02	-28.81	1.73 ±0.01	1.53 ±0.01	-11.18
Winter	21.96 ±0.01	16.29 ±0.015	-25.77	19.51 ±0.01	15.48 ±0.016	-20.67	1.95 ±0.01	1.66 ±0.015	-14.70

*Average values of triplicate samples

POC: Percent over control

± Standard deviation

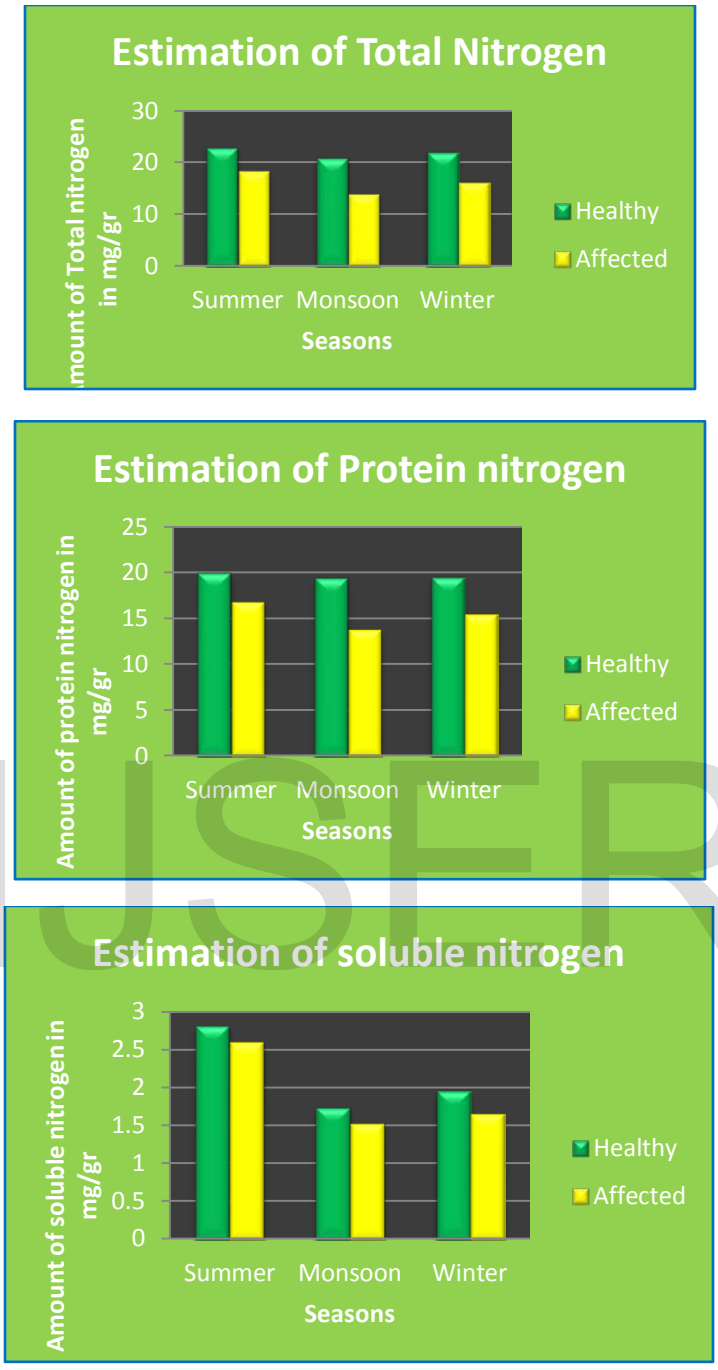

Fig-1: Bar diagrams showing the changes in nitrogen fraction of healthy and affected *S. cumini* leaves

Table – 2: Total lipid composition of healthy and *C. filiformis* affected *S. cumini* leaves at different seasons of infection.

Seasons	Total lipids (mg/g)			Total glycolipids (mg/g)			Total phospholipids(mg/g)		
	S. cumini			*S. cumini*			*S. cumini*		
	Healthy	Affected	POC	Healthy	Affected	POC	Healthy	Affected	POC
Summer	245.35 ±0.01	199.176 ±0.015	-18.81	2.33 ±0.02	1.253 ±0.015	-46.28	612.44 ±0.01	551.33 ±0.015	-9.97
Monsoon	204.84 ±0.01	129.21 ±0.015	-36.92	2.063 ±0.01	1.103 ±0.01	-46.50	582.36 ±0.02	525.96 ±0.02	-9.68
Winter	243.25 ±0.01	152.43 ±0.01	-37.34	2.126 ±0.001	1.174 ±0.02	-44.78	621.13 ±0.015	573.92 ±0.02	-7.60

*Average values of triplicate samples
POC – Percent over control
± Standard deviation

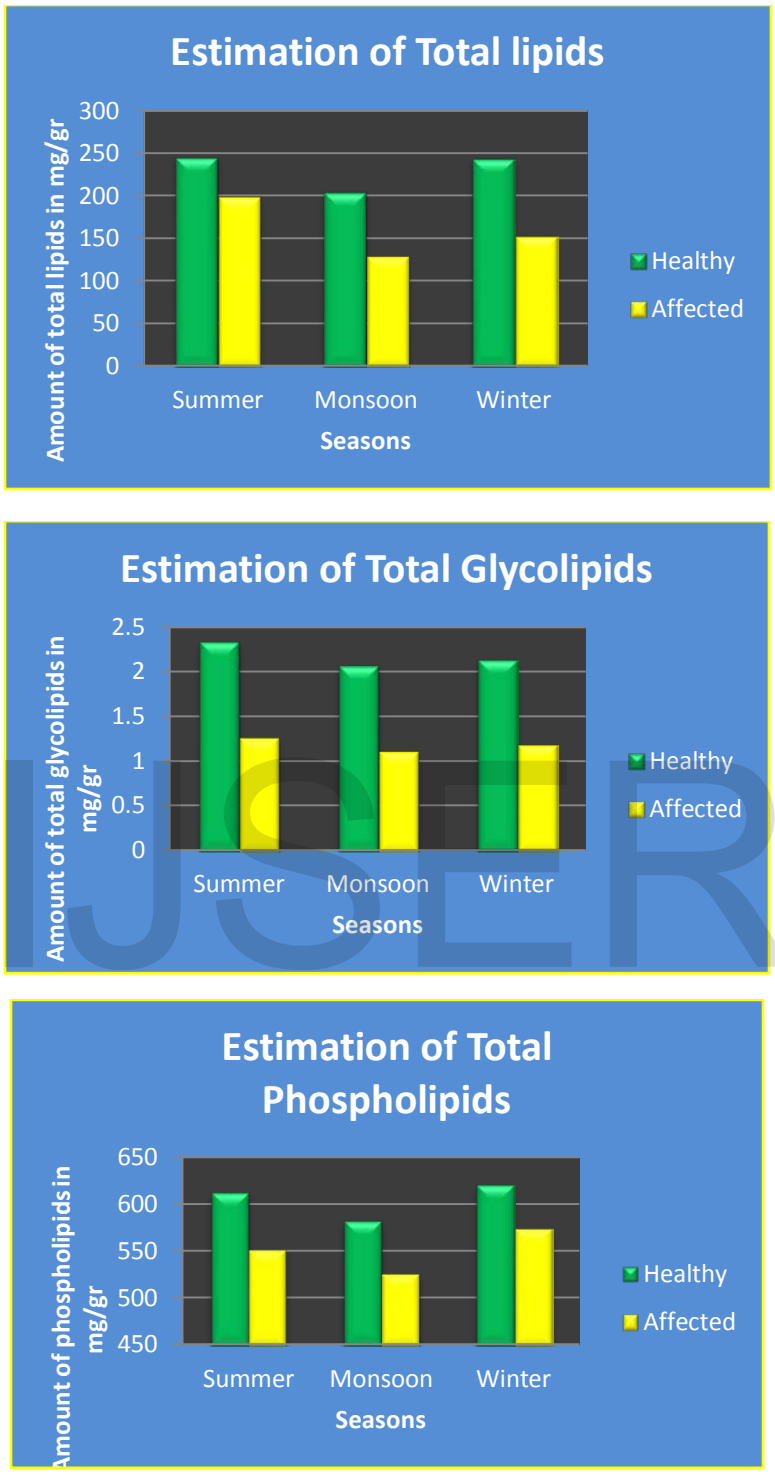

Fig-2: Bar diagrams showing the changes in lipid composition of healthy and affected *S. cumini* leaves

In the present study, some metabolites like Nitrogen and Lipids in infected leaves of the host *S. cumini* by *C.filiformis* was compared with healthy host leaves. Significant differences were observed in the two metabolite parameters.

The total nitrogen and protein nitrogen content was high in healthy leaves of *S. cumini*, while it is less in affected leaves of *S. cumini*. Soluble nitrogen is higher in the healthy leaves and lesser in the affected leaves. Similar trends were observed in the concentrations of nitrogen and phosphorus was observed in leaves of infected plants compared to control plants (Hibberd *et al*., 1996).

Lipid composition in healthy *S. cumini* leaves is high when compared to affected leaves in all the three seasons. Total glycolipids and phospholipids in healthy leaves of *S. cumini* were high when compared to affected leaves of *S. cumini* in all three seasons investigated. The results are in agreement with the earlier reports. In the biochemical studies of parasitism of mistletoes, (Suryaprakash *et al*., 1967) on the studies of the leaves of *Dendrophthoe falcate* (*Loranthus* parasite) growing on different trees had contained accumulated phosphates in excess of host leaves. The present studies did not analyses the parasite leaves as the *C. filiformis* is a leafless plant. Phospholipids were higher in host leaves than in parasite leaves (Suryaprakash *et al*., 1967). Gil and Hawksworts (1961) quoted that ash from twigs of *Viscum album*, contained more phosphorus than the ash from host twigs. Michel-Durand (1934) investigated the seasonal changes in the organic and inorganic phosphorus in the leaves *of Viscum album* where they found that lipid phosphorus remained constant from May to September, phytic acid and mineral phosphorus decreased, with a reciprocal increase in organic phosphorus under France condition.

CONCLUSION

From an overall consideration of the results of the affected plants it may be concluded that *C. filiformis* interferes with various metabolites biosynthesis mechanisms of the host plant for the benefit of the parasite growth and reproduction, thus reducing the yield potentials of the host plant.

From the above results of the investigation it was clear that the parasite *C. filiformis* continuously absorbing the host plant's nutrients and metabolites namely total nitrogen, protein nitrogen, soluble nitrogen,

lipids, total Glycolipids, phospholipids. Studies on control mechanisms of the parasite with biological and non – harmful herbicides will further help in the management of the parasitism on host plantations.

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