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Studies of biochemical changes in healthy cowpea plants mechanically infected with sap isolated from virus infected *Clerodendrum inerme* leaves.

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ABSTRACT
Clerodendrum inerme L. Gaertn belongs to family Verbenaceae,is commonly known as Sankuppi (Glory bower, Garden quinine) in Uttar Pradesh. Its hardy nature and the closely held branches and leaves,promoted itto a garden plant. Clerodendrum plants showing symptoms suggestive of viral infection such as leaf roll, mosaic, chlorotic spots, yellowing and leaf distortion were visually inspected. Present study makes a comparative analysis of nutrient contents of cowpea (Vigna unguiculata), infected with sap obtained from virus infected C. inerme leaves. Protein, total nitrogen and pigment contents were analyzed from both control and mechanically infected cowpea plants, collected 30 days after inoculation. Virus infection reduced the productivity as found loss in pigments and resulted in increase in total nitrogen and protein as compared to healthy ones.
Keywords: *Clerodendrum inerme,* Pigment content, Protein contents

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INTRODUCTION

Clerodendrum inerme belonging to Family *Verbenaceae*, is a very common ornamental hedge plant found in Indian gardens. It is an important medicinal plant used for the treatment of asthama, elephantiasis and skin burns. Juice of *C. inerme* is taken orally to relieve stiffness of legs and muscular pains (2). Plants exhibited yellow green mosaic symptoms, reduction in leaves size and retarded plant growth. *C. inerme* plants are used as hedge in Dayalbagh Educational Institute campus. External appearance of symptoms was suggestive of viral disease, which was later confirmed through electron microscopy (Figure 1). Cowpea plants were chosen as propagative host, as it was most susceptible for the viral multiplication, easy to grow and maintain in green house conditions. Cowpea is an important legume crop cultivated in all part of India and worldwide for green pods as vegetable, seeds as pulse (7).

Figure 1. Leaves of *Clerodendruminerme* showing disease

MATERIAL AND METHODS

Experimental setup- Mechanical sap transmission was used for transmission of virus from diseased leaves of *C. inerme* to host plants.Virus inoculum was prepared from infected leaves of *C. inerme* using 0.1 M sodium phosphate buffer with celite, used as abrasive. Seeds were sown in separate pots and transmission was done at 4-6 leaves stage.

(A) Selection of suitable propagative host- Mechanical transmission was used for the selection of host, *Lageneria siceraria, Cucumis melo, Luffa cylindrica, Cucurbita maxima, Cucumis sativus, Nicotiana sps.,Lycopersicon esculentum, Vigna radiata, Vigna unguiculata, Vigna mungo, Achyranthus aspera* were used.

(B)Study of biochemical changes in cowpea plants

(i) Inoculation- Two lots of 20-20 plants were taken. One lot of plants was inoculated with sap of infected *C. inerme* leaves and second lot was kept as control.

(ii) Protein contents- Protein contents were determined by using spectrophotometer procedure used by 4, 6. 5 gms of dried plant material (leaf, stem and root) was crushed with 10 ml of 20 % trichloroacetic acid. Homogenate was centrifuged at 6000 rpm for 15 min. To the residue, 5ml of 0.1N NaOH was added and centrifuged. The supernatant was collected and made to 5ml with 0.1N NaOH. From the extract, 0.5 ml of the sample was taken in a testtube and 5 ml of alkaline copper solution was added. 0.5 ml of folin-phenol was added with vigorous mixing. The mixture was kept in dark for 30 min. The sample was read at 660 nm in spectrophotometer. Blank was prepared without protein sample. Standard graph of protein was prepared by using 5th fraction of Bovin Serum Albumin. The amount of protein was determined by multiplying its Kjeldahl nitrogen content by a factor of 6.25. The amount of protein was expressed as mg/g.

(iii)Total nitrogen -Total nitrogen was estimated by method followed by 5. In each treatment the material to be treated was taken from five plants. This material was dried at room temperature for 72 hours. 2 g of powdered sample was digested in a Kjeldahl digestion flask by boiling with 20 ml of concentrated H_2SO_4 and a Kjeldahl digestion tablet (catalyst) until the mixture was clear. Ammonia was steam distilled from of the digest to which 50 ml of 45% sodium hydroxide solution was added. 150ml of the distillate was collected in a conical flask containing 100ml 0.1N HCl and methyl red indicator. The ammonia that distilled into the receiving conical flask, react with the acid and the excess acid in the flask was estimated by back titration against 2.0M NaOH with colour change from red to yellow (end point). Determination was made on all reagents alone (blank determinations).

$$\% \text{ Nitrogen} = \frac{(\text{ml standard acid x N of acid}) - (\text{ml blank x N of base}) \text{ x } 1.4007}{\text{Weight of sample in grams}}$$

Where N=normality

(iv) Pigment content- Estimation of pigment was done by procedure followed by 1, 3. 0.5 ml of sap each from healthy and symptomatic plants of the same age was mixed with 4.5 ml of water and 20 ml acetone. Each mixture was filtered through whatman no. 1 filter paper and the optical density of the clear solutions was determined by spectrophotometer at different lengths. The values of chl a, chl b and total chlorophyll were determined by the following expressions:

Chlorophyll a $(\frac{mg}{l})$ = 15.6 OD_{665}-2.0 OD_{645}-0.8 OD_{630}

Chlorophyll b (mg/l) = total chlorophyll- chlorophyll a

Total chlorophyll (mg/l) = OD_{652} x $\frac{1000}{34.5}$

RESULTS AND DISCUSSION

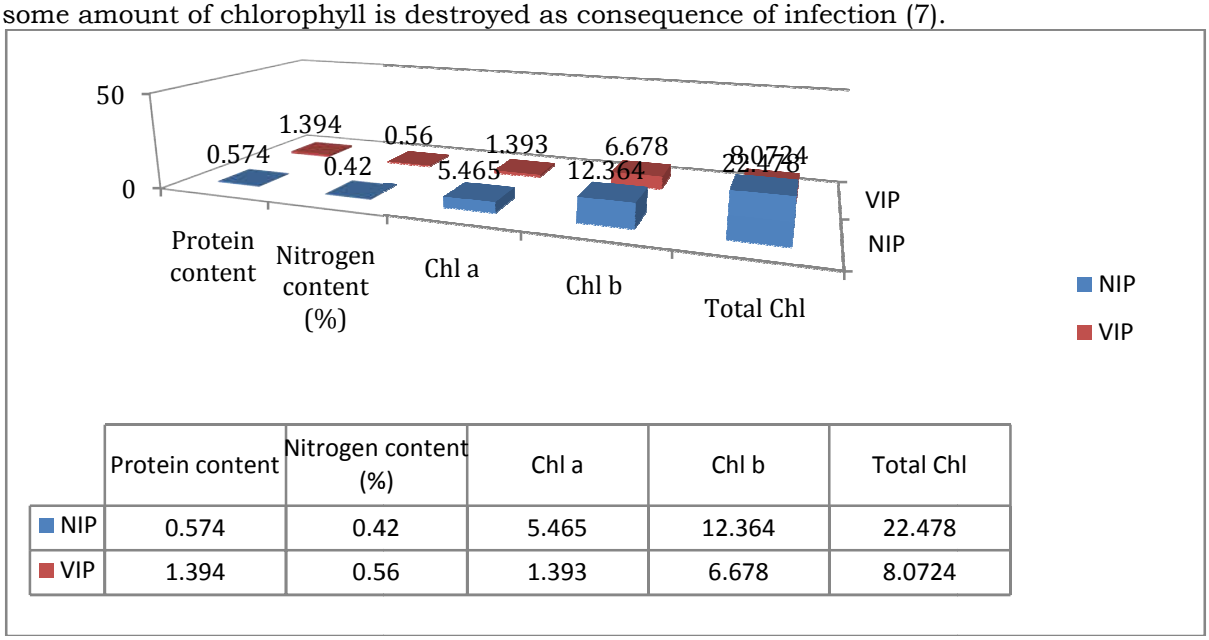
(A)Selection of suitable propagative host-Mechanical inoculation was successful for the transmission of virus, as symptoms were observed on plants. Plants were observed at regular intervals after three days. Of the 11 host plants tested, for mechanical transmission, *Vigna unguiculata* was found to be most suitable. Symptoms in cowpea were varied and included mosaic, puckering, mottling along with vein clearing, reduced leaf size, green spots, and depressions on leaf, chlorotic spots etc.(Figure 2).

a b c

Figure 2. Image A showing leaf of Cowpea used as control, B and C showing symptoms reduced leaf size, green spots, and depressions on leaf, chlorotic spots etc

(B) Study of biochemical changes in cowpea- In non-infected plants (NIP), protein contents was found to be 0.574 mg/g and Nitrogen content was 0.42%, total chlorophyll was 22.478 mg/l whereas in virus infected plants (VIP), there was a dramatic increase in protein contents was 1.394 mg/g and nitrogen was estimated to be at 0.56%. However, total chlorophyll was reduced to 8.0724 mg/l (Figure 3).

Thus it was observed that the infection caused by virus led to the increase in the total protein and nitrogen content of the host plant leaves. The proteins are complex polymer of amino acid with high molecular weight. Infection of virus in plants should be involved in the protein metabolism of the host cells. Decrease in chlorophyll content is due to chlorosis and necrosis of diseased plant parts. Chlorophyll is the most important component of the photosynthetic system. In plants, virus infection includes change in the colouration of leaves, virus infection frequently involves the colour change in most of the plants shows that chlorophyll content is either not synthesized at the same rate as in healthy plants or some amount of chlorophyll is destroyed as consequence of infection (7).



	Protein content	Nitrogen content (%)	Chl a	Chl b	Total Chl
NIP	0.574	0.42	5.465	12.364	22.478
VIP	1.394	0.56	1.393	6.678	8.0724

VIP- Virus infected plants, NIP- Non-infected plants

Figure 3. Change in protein, nitrogen content and pigments in healthy and diseased leaves of Cowpea infected with *Clerodendrum inerme* associated virus.

CONCLUSION

Studies of biochemical changes in healthy cowpea plants mechanically inoculated with sap isolated from virus infected *Clerodendrum inerme* leaves revealed increase in protein and nitrogen content, and drastic decrease in total chlorophyll.

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