

## Characterization of virus causing chlorotic spot disease in Garden quinine and its effect on nitrogen and pigments

SONAL AND SHARMITA GUPTA

Department of Botany, Faculty of Science, Dayalbagh Educational Institute (Deemed University) Dayalbagh, Agra (U.P.)-282 005

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### ABSTRACT

The greenhouse studies were carried out at D.E.I., Dayalbagh during 2014-16 to characterize virus causing Chlorotic spot disease in garden quinine (*Clerodendrum inerme* L. Gaertn) and its effect on nitrogen and pigments. The results revealed that more than 85% plants (86.3% during 2014-15 and 90.3% during 2015-16) were infected with chlorotic spot disease caused by virus. In green house studies, out of 52 host plants, 41 plants were found susceptible and showed positive response for the symptoms. Nitrogen and protein contents were markedly higher in diseased leaves and stems than those of healthy plants. The contents of plant pigments namely chlorophyll a, b and total chlorophyll decreased in diseased leaves as compared to healthy one. The extents of reduction in these pigments were 44.46, 40.00 and 50.23 per cent, respectively.

**Keywords:** Garden quinine, viruses, mosaic and nitrogen etc.

### INTRODUCTION

In present time, viruses are responsible for a number of diseases in different plants. Plant viruses are causal agents for severe losses and extensive damage to the plants. Garden quinine plants were suspected for the disease caused by virus on the basis of symptoms like mosaic, leaf rolling, chlorotic spots etc. Garden quinine (*Clerodendrum inerme* L. Gaertn) is a perennial shrub, grown as hedge plant in parks and home gardens. Garden quinine is valued for landscaping as a bonsai, topiary and hedge plant. The plants are trimmed to form figure of birds, animals and geometrical shapes in the parks and gardens (Parmaret *et al.*, 2013). It is evergreen sprawling shrub 1-1.8 m tall, woody stems and smooth with ovate to elliptical (5-10 cm) long leaves. It has attractive evergreen foliage and fragrant white flowers that form in clusters and are accented by delicate red protruding stamens. Disease produced by any pathogen in plants, affects production and supply of nutrients, essential for growth of plants. Scanty information is available on infection of Chlorotic spots disease in garden quinine in Agra. The present study aims to investigate the disease prevalence of *Clerodendrum inerme* yellow mosaic in Agra region along with biological characterization.

### MATERIALS AND METHODS

#### Maintenance of virus culture

Sap extracted from the symptomatic leaves of garden quinine was inoculated to test plants. Test plants showing best systemic symptoms were used for maintenance of virus culture. Diseased garden quinine was used as stock culture. Symptomatic leaves of garden quinine, hedge present in Dayalbagh Educational Institute, Dayalbagh, were collected and washed with water. Extract from symptomatic leaves of garden quinine was obtained by maceration in 0.1M Sodium phosphate buffer having pH-7.0, separately. For the presence of garden quinine mosaic symptoms, survey was conducted in the different areas of Agra for the visual inspection of the symptoms produced by the viral infection. From surveyed areas, diseased and healthy plants were counted per location to know about disease incidence. Disease incidence was calculated as per the formulae:

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{No. of total plants assessed}} \times 100$$

Biological characterization include infectivity assay through sap inoculation, vector and graft transmission for variations in

symptoms expression and host range studies (Hill, 1984). For the estimation of protein and nitrogen, stem and leaf samples were collected separately from healthy and infected garden quinine plants. The total nitrogen was measured by the modified micro kjeldahl method (Lang, 1958) and total protein content was determined by multiplying the total nitrogen content with 6.25. Photosynthetic pigments were also studied following the procedure used by Pande (2017). Statistical analysis was carried out using Microsoft ORIGIN software.

## RESULTS AND DISCUSSION

### Incidence of chlorotic spot disease

Garden quinine plants showed symptoms suggestive of viral infection, such as leaf roll, mosaic, chlorotic spots, yellowing and leaf distortion were visually inspected (Fig. 1). Symptomatic and symptomless plants were counted as per areas visited from different sites and it was found that 86.34 % disease incidence was observed in year 2014-15, whereas it increased to 90.34 % in year 2015-16.



Fig.1A Diseased Garden quinine leaves showing symptoms viz. mosaic, chlorotic spots; Fig. B. inward leaf rolling and distortion in diseased leaves

### Biological Characterization

Out of 52 host plants, 41 host plants were found susceptible and showed response for the symptoms expression. Viral nature of the symptomatic garden quinine was successfully established by sap inoculation method. The symptoms on the indicator plants such as *Lageneria siceraria*, *Cucumis melo*, *Luffa cylindrica*, *Cucurbita maxima*, *Cucumis sativus*, *Nicotiana sps.*, *Lycopersicon esculentum*, *Vigna radiata*, *Vigna unguiculata*, *Vigna mungo*, *Achyranthus aspera* varied from plant to plant. These included mosaic, puckering, mottling along with vein clearing, reduced leaf size, green spots, depressions on leaf, chlorotic spots etc. Any symptom produced by virus was observed on host plants infested by whiteflies and aphids collected from fields and inoculated for 15 mins to the diseased leaves of garden quinine. Plants were maintained in greenhouse for one month but vector transmission through whiteflies was successful and host plant showed

chlorotic spots on the leaves where flies were fed after acquisition, whereas no symptoms were produced by aphids. Wedge grafting was successful for the graft transmission study of *Clerodendrum* associated mosaic virus

Table 1: Effect of virus infection on nitrogen and protein content (%) in garden quinine

Plant parts/Parameter	Nitrogen (%)	Protein (%)
Healthy leaf	0.42±0.025	2.62±0.15
Diseased leaf	0.66±0.03	4.12±0.18
Healthy stem	0.20±0.07	1.25±0.43
Diseased stem	0.31±0.06	1.93±0.37

### Nitrogen and pigments

In the present study it was observed that the virus infection increased nitrogen and protein content of the host plant in symptomatic leaves and stems as compared to healthy one (Table 1). Loss in chlorophyll content was observed in diseased leaves of garden quinine. The per cent

decrease was evaluated and recorded loss in chl a (44.46%), chl b (40%) and total chlorophyll (50.23%) (Table 2 Fig. 1). Experiments were performed in triplicates.

Table 2: Effect of virus infection on pigments (mg/l fresh weight of leaf tissue) in garden quinine

Pigment	Healthy leaf	Diseased leaf	% Decrease
Chlorophyll a	5.87±0.39	2.61±0.07	44.46
Chlorophyll b	3.85±0.62	1.54±0.36	40.00
Total chlorophyll	10.63±0.93	5.34±0.60	50.23

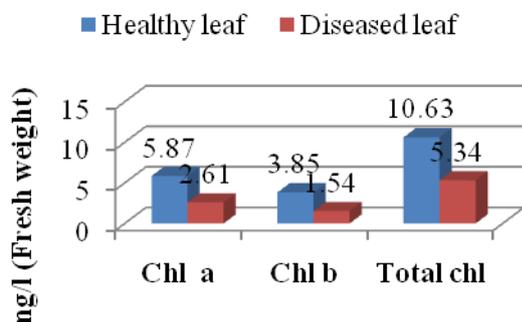


Fig.1 Effect of virus infection on pigments (mg/l fresh weight of leaf tissue) in garden quinine

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Is has been observed that nitrogen content was higher ( $0.66\pm 0.03$ ) and ( $0.31\pm 0.06$ ) in diseased leaves and stems of garden quinine, when compared to healthy ones ( $0.42\pm 0.025$  and  $0.20\pm 0.07$ ) in leaves and stems, respectively. Nitrogen plays vital role in the nutrient correlation between plants and pathogens. The increase in nitrogen may be probably due to the accumulation of free amino acids as a result of the hydrolysis of host proteins (Pande 2017). Nitrogen enhances the rapid growth; improve protein content of plant. Deficiency of nitrogen causes reduced growth, appearances of chlorosis (Bianco *et al.*, 2015). Whereas increase in protein levels could be contributed to the types of proteins synthesized by the host, as affected by virus. Chlorophyll pigments are necessary in the plants for the manufacture of carbohydrates which form the basis of all foods for both plants and animals. It is for this reason that chlorophyll pigments are regarded among the most important chemical substances in nature. Similar observations were observed by (Meena, 2016).