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Effect of Cowpea Golden Mosaic Virus infection on Rhizobium in nodules of cowpea (*Vigna unguiculata*)

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Abstract
Cowpea is an important leguminous crop cultivated in all part of India and worldwide for green pods as vegetable, seeds as pulse. Being leguminous crop the roots of plants have nodules which help in nitrogen fixation and improving fertility of soil. Virus infection adversely affects all the parts of plant including nodules of roots. Present study deals with the leghaemoglobin content and number of Rhizobium in root nodules of Cowpea Golden Mosaic Virus infected plant, it was found that infected plant's nodule have lowered amount of leghaemoglobin content and they harbor less number of bacteria when compared with healthy control plants and this adversely affects nitrogen fixation by infected plant.
Keywords: Cowpea, Leghaemoglobin Rhizobium, Virus infection and nitrogen fixation.

Introduction
Members of the *Rhizobium* have been found to penetrate the root hairs of leguminous plants and ultimately give rise to a small ball like structure known as nodule. Pink colour of nodule is due to presence of leghaemoglobin which provides anaerobic condition for fixation of nitrogen. Nodules are related with atmospheric nitrogen fixation which may affect directly or indirectly the nitrogen content of the soil and the plant. Since the virus infected host plants exhibit a change in their nitrogen content [1], it may affect the morphology of nodules of their hosts. The nitrogen economy acquired by nodules and its bacterial population through symbiosis in the leguminous plants depends upon the biochemical reactions taking place inside the nodules, involving leghaemoglobin, Peroxidase and catalase activities [2]. The present investigations study the effect of virus infection on leghaemoglobin content and *Rhizobium* population in the nodules of infected cowpea plants.

Materials & Methods-
Virus inoculum and inoculation procedure:
Young infected leaves of vigna unguiculata c.v. Pusa Komal with distinct virus symptoms were collected from surveyed field and used as food for the whiteflies. 2cm wide and 5cm. long straight glass tube whose one end in connected with rubber tubing with a cloth barrier between the glass and rubber was used as aspirator to collect whiteflies by sucking through rubber tubing . These white flies were allowed to feed on infected leaves for 12 hours.
After feeding whiteflies were collected carefully and placed on test seedings plants for 24 hours. About 10 whiteflies per plants were used for the transmission. Test plants were inoculated when first trifoliate emerged. 0.2% Imidachloprid insecticide was used to kill the white flies. Test plants which were not inoculated with white flies served as control and kept under observations [3].
Leghaemoglobin is responsible for the pink colour of nodules and is related to the activity of the nitrogen fixation in the nodules, which is economically important for maintaining fertility without external use of fertilizer [4].
An experiment was conducted to see the effect of CPGMV infection on leghaemoglobin content and Rhizobium population of nodules of cowpea plants.
Estimation of Leghaemoglobin:
In this experiment the leghaemoglobin content in nodules of cowpea plants infected with CPGMV has been estimated at 20, 30, 40, 50 and 60 days after inoculation using the method described by Tu. ford and Grau [5]. Nodules from healthy and diseased plants at different intervals were harvested by removing the soil in running water through a 30 mesh screen and collecting the nodules from the screen. Those still were adhering to the roots were picked off individually. Nodules were washed several times with water and surface dried with paper towel and stored in a plastic bag. It was then frozen before leghaemoglobin extraction.

One gram nodules were homogenised in 5 ml of 0.1N KOH and centrifuged for 10 minutes at 12,000 x g; in 1.5ml of supernatant 1 ml of distilled water; 0.5ml of 5N KOH, 0.1g of $Na_2S_2O_4$ was added for reduction. The optical density of the mixture was determined at 537, 557 and 577nm by Carl Zeiss Jena Spectro colorimeter, 10 minutes after mixing. Using a blank of the above mixture, without the nodule extract the value of leghaemoglobin was calculated by the following formula:

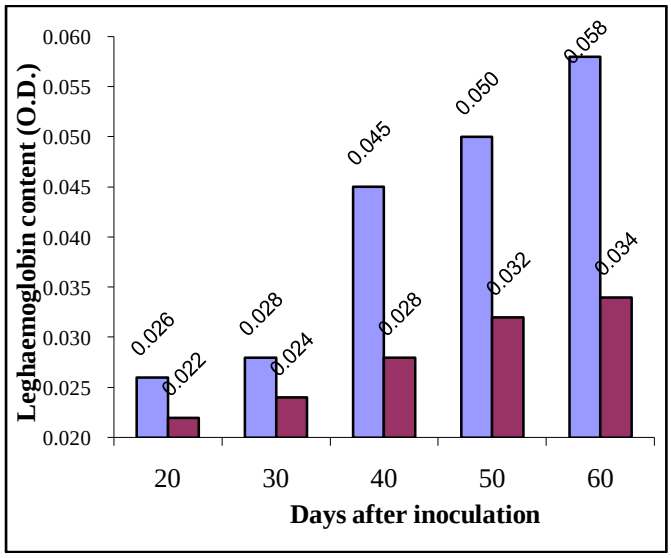
Leghaemoglobin content in O.D. = $OD_{557} - ½ (OD_{537} + OD_{577})$

Table 1: Effect of CpGMV infection on Leghaemoglobincontent in nodules of *vigna unguiculata*

Days after inoculation	Leghaemoglobin Content (OD)		Percent decrease
	Healthy root nodule	Infected	
20	0.026	0.022	15.38
30	0.028	0.024	14.28
40	0.045	0.028	37.77
50	0.050	0.032	36.00

60	0.058	0.034	41.37
Average	0.0414	0.028	28.96

Figure 1: Effect of Cowpea golden mosaic virus infection on Leghaemoglobin content of nodules of the *Vigna unguiculata*



Rhizobium Culture-

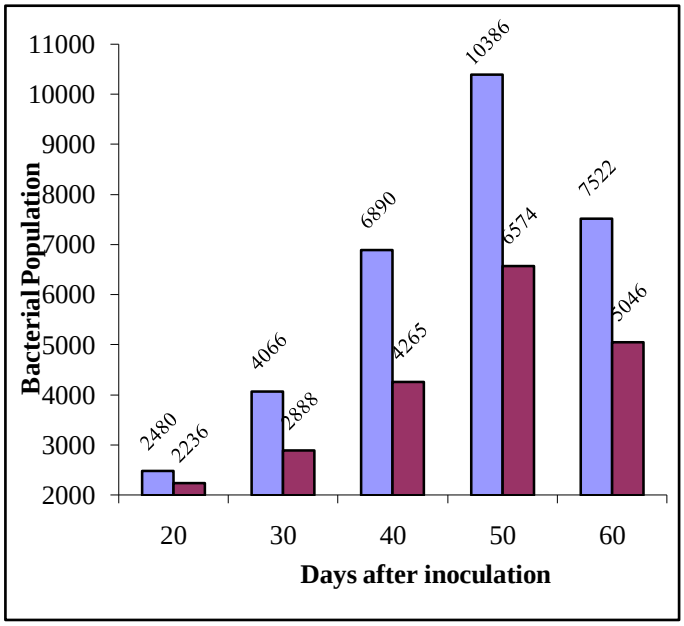
The nodule bacterial was cultured on yeast- mannitol agar medium, pH of the medium was maintained at 7.0. The medium were sterilized at 15 lb pressure and 120^0C temperature for 15 min. The glassware was sterilized for 2h at 170^0 to 180^0C in a hot air oven.

Fresh nodule samples were collected from healthy and diseased cowpea roots; then roots were washed thoroughly with distilled water. One hundred mg of nodules sample were homogenised with 100ml distilled water in a mortar. This was further diluted to one hundred times and 1 ml of diluted suspension was transferred in each of 5 petridishes, after pouring the medium cultures were incubated at 25^0C for 24 hrs., Bacterial colonies were then counted and their population calculated as number of bacterial colonies per gram of nodule.

Table 2: Bacterial population (Colony count/g) of nodules of *vigna unguiculata* at different periods of CpGMV infection

Days after inoculation	Bacterial Population		Percent decrease
	Healthy root nodule	Infected root nodule	
20	2480	2236	9.83
30	4066	2888	28.97
40	6890	4265	38.09
50	10386	6574	36.70
60	7522	5046	32.91
Average	6268.8	4201.8	29.3

Figure 2: Effect of Cowpea golden mosaic virus infection on bacterial population of nodules of Vigna unguiculata



Discussion :

A general increase is evident from the table-& fig-1 in leghaemoglobin content of both healthy and infected nodule samples with the age of the plants. It was found that nodules of healthy plants had higher leghaemoglobin content than those of diseased ones.

Nutman [6] believed that the pigment leghaemoglobin was responsible for the pink colour of nodules and was related to the activity of nitrogen fixation in nodules. Jordan [7] reported that leghaemoglobin is essential in the nitrogen fixation. However, the effect of virus on nodular leghaemoglobin is little known.

Joshi *et al.* [8] observed that the healthy white clover produced large pink root nodules. Tu. Ford and Grau [5], however, did not observe such striking colour differences in nodules of healthy and Soyabean mosaic virus infected Soybean plants and they reported that the nodules of healthy soybean plants had the higher leghaemoglobin content than those of Soybean mosaic virus diseased nodules.

Rajgopalan and Raju [9] found that the formation of leghaemoglobin in nodules of *Dolichos lablab* was not affected by the Dolichos enation mosaic virus infection. The peak concentration of pigment was, however, attained earlier in nodules of infected than in healthy ones.

Srivastava [10] also observed lesser amount of leghaemoglobin in sesbania infected with Sesbania mosaic virus. Tripathi (1985) observed similar finding in Cowpea vein banding virus infected cowpea plants.

Rao *et al.* [11] and Rao and Shukla[12] reported lesser leghaemoglobin content in pea root nodules infected with cucumber mosaic virus and sesbania mosaic virus, respectively.

Sharma and Varma [13] observed similar results, wherein leghaemoglobin content was reduced by Cowpea chlorotic spot virus infection of cowpea plants.

Patil and Sayyad [14] under took the study of leghaemoglobin content in cowpea nodules as influenced by virus-rhizobium interactions. They reported that virus infection reduced the leghaemoglobin content substantially.

In a similar study on *Vigna sinensis*, grown in three different culture media, Upadhyaya *et al*. [15] found that Cowpea vein banding virus affected the leghaemoglobin content adversely.

The decrease in leghaemoglobin content in nodules of virus infected cowpea plant is probably due to metabolic disturbance in the host plant caused by CPGMV infection. It is also possible that interaction between the virus and *Rhizobium* might have lowered the leghaemoglobin content.

Patil and Sayyad [14] reported that virus - *Rhizobium* interactions have reduced the leghaemoglobin content in the nodules of cowpea plant.

The findings of the present study reveal that the bacterial population of CPGMV infected cowpea nodules were always less than healthy ones Table 2 & Figure 2, similar findings have been observed by Srivastava [10] in sesbania infected with Sesbania mosaic virus and in pea infected with CMV and Sesbania mosaic virus [11] Rao *et al* , Rao & Shukla [12] and in Vicia faba infected with Bean mosaic virus Rao et al [16].

The overall metabolic alterations brought in by virus in the infected nodules seem to be cause for low rate of multiplication of *Rhizobium*.

Manil [17], reported that increase in the bacterial population with the age of the plant is possibly due to genetic properties of the host and bacteria. Heumann [18] observed a positive correlation between the percentage of bacteroids and the intensity of leghaemoglobin in reaction.

Rajgopalan and Raju [9] were opinion that increase or decrease in nodule number might be associated with the physiological status of the root and density of rhizospheric micro-organisms. Effect of Mungbean mosaic virus on nodulation in *Vigna radiata* [19]. showed physiological alteration in virus infected roots associated with decreased bacterial population is noticed. Thus it may be possible that change physiological status of the root associated with low bacterial population of infected plants

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