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## Effect of geminivirus infection on nodulation in cowpea

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### ABSTRACT

Cowpea is an important leguminous crop which also helps in improving soil quality by fixing nitrogen with the help Rhizobium. In roots symbiotic association with Rhizobium occur which results in nodule formation. In plants infected with viruses nodule formation is also affected. In this paper effect of geminivirus infection on physical attributes of nodule formation like number, weight and volume is studied and found to be adversely affected due to infection caused by geminivirus on cowpea. Virus infection has influenced the nodulation ability in cowpea. Nodules of infected cowpea plants have reduced number of nodules, decreased fresh weight and volume than their comparable healthy plants. The percentage of reductions was increased with the age of the plants. Number, fresh weight and nodules also increased with increased age of the plants both in healthy and infected plants.

**Keywords-** Nodulation, Cowpea, Geminivirus

### INTRODUCTION

Cowpea *Vigna unguiculata* (L) walp is native of Central Africa since wild forms are found only there, from where it spread in early times through Egypt or Arabia to Asia and the Mediterranean. Grain legumes are important constituents of vegetarian diet in India. Among the grain legumes, cowpea is one of the important pulse crop. Cowpea is also cultivated for fodder, green manure, vegetable, and soil improving cover crop. Legume-rhizobia symbiosis results in the fixation of the atmospheric nitrogen in the soil. For those species of plant, which are capable of utilizing atmospheric nitrogen for growth, this provides a much cheaper source of N than nitrogenous fertilizer. 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. 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 [5] it may affect the morphology of nodules of their hosts. Like other valuable crops cowpea is also subjected to various diseases among which virus disease occupy an important place as they cause great loss in the yield. Among the 34 viruses, reported to infect cowpea, in India it is infected by at least 16 different viruses [22] The present work evaluates the effect of virus inoculation on nodulation in cowpea.

### MATERIALS AND 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 seedlings plants for 24 hours. About 10 whiteflies per plants were used for the transmission. Test plants were inoculated when first trifoliolate 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.

#### Experimental Conditions -

The experiments in the present study were carried out in an insect proof chamber, where usual precautions were taken to keep the plants free from insects and nematode infection.

The nodules of healthy and infected cowpea plants were sampled on 20, 30, 40, 50 and 60 days after their germination. Due care was taken not to lose even a single nodule during uprooting and washing of the roots for the nodule collections. After washing and blotting, the nodules were immediately counted, weighed and volumetrically measured using measuring cylinder, water and sinker. Nodules were always stored in a polythene bag, so as not to lose moisture during their observations. The number of nodules was given per plant, while the volume in cc and weight in gram were given per nodule. The results were taken as an average of 30 plants. Percentage was also calculated based on the values obtained for healthy samples. The experiments were repeated three times and an average of results is presented in tables 1 to 3

### RESULTS AND DISCUSSION

The results presented in Table-1 to 3, Plate 1 & Figs. 1 to 3 indicates that virus infection has influenced the nodulation ability in cowpea. Nodules of infected cowpea plants has reduced number, fresh weight and volume than their comparable healthy plants. The percentage of reductions was increased with the age of the plants. Number, fresh weight and nodules also increased with increased age of the plants both in healthy and infected plants.

**Table-1: Effect of CpGMV infection on nodulation of cowpea  
(Changes in number of nodules/plant)**

Days after inoculation	Average number of nodules/plant		Percent reduction
	Healthy	Infected	
20	78.3	59.2	0.011
30	99.1	74.3	0.0121
40	111.3	80.7	0.0141
50	114.3	81.7	0.0155
60	118.2	82.5	0.0179
<b>Average</b>	<b>104.24</b>	<b>75.68</b>	<b>0.01412</b>

**Table-2: Effect of CPGMV infection on fresh weight of nodule of cowpea**

Days after inoculation	Average fresh weight/nodule in mg.		Percent reduction
	Healthy	Infected	
20	0.011	0.01	9.09
30	0.0121	0.011	9.09
40	0.0141	0.0117	17.02
50	0.0155	0.0125	19.35
60	0.0179	0.014	21.78
<b>Average</b>	<b>0.01412</b>	<b>0.01184</b>	<b>15.266</b>

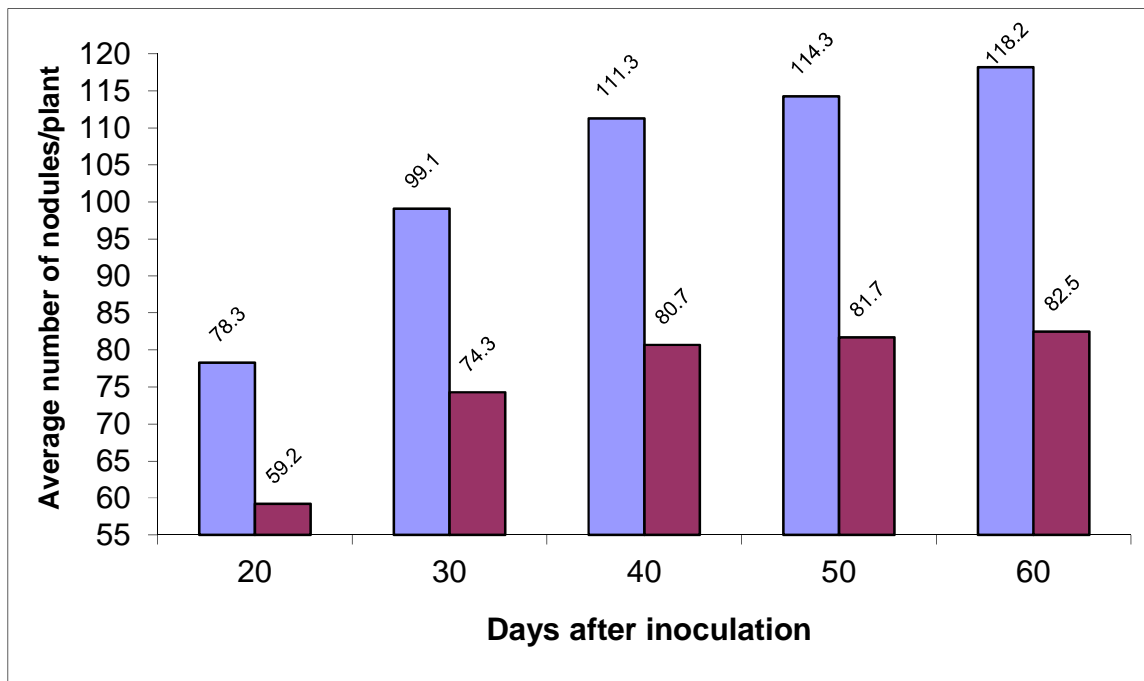


Figure 1: Effect of Cowpea golden mosaic virus infection on changes in number of nodules/plant

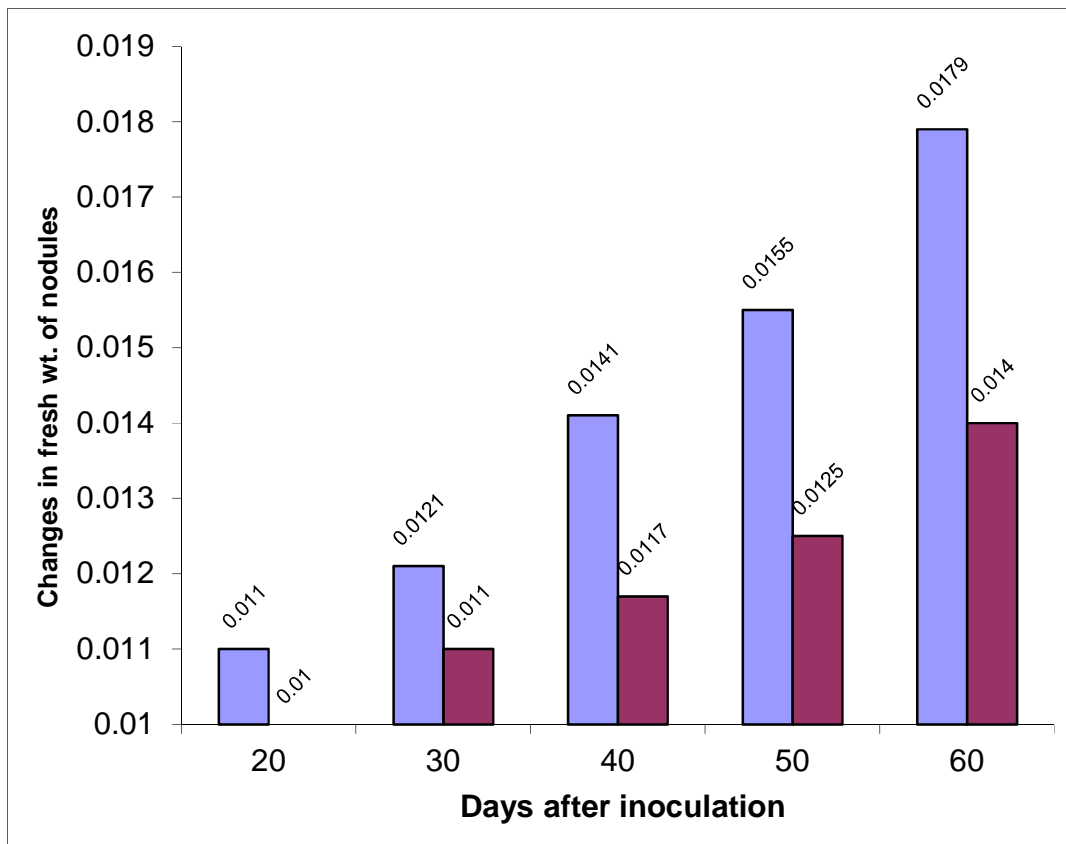
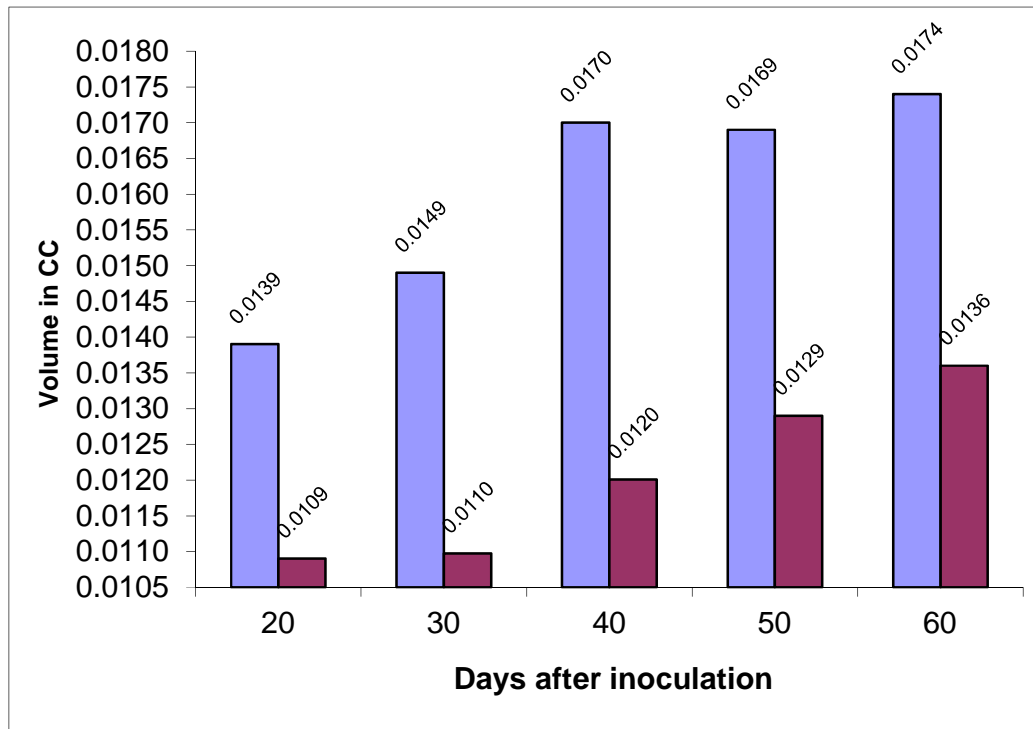


Figure 2 : Effect of Cowpea golden mosaic virus infection on change in fresh weight of nodules of *Vigna unguiculata*

Table-3 :Effect of CpGMV infection on changes in volume of nodules of cowpea

Days after inoculation	Average volume/nodule in CC		Percent reduction
	Healthy	Infected	
20	0.0139	0.0109	21.58
30	0.0149	0.01097	26.37
40	0.017	0.01201	29.35
50	0.0169	0.0129	33.66
60	0.0174	0.0136	34.83
<b>Average</b>	<b>0.01602</b>	<b>0.0120</b>	<b>29.158</b>

Figure 3 : Effect of Cowpea golden mosaic virus infection on volume of nodules *Vigna unguiculata*

Reduction in nodule number due to virus infection has also been reported in soybean infected with Soybean mosaic virus and Bean pod mottle virus [8] Rajgopalan and Raju, [12] noticed reduction in root nodule development in *Dolichos lablab*, was directly related to the degree of infection by Dolichos enation mosaic virus in inoculated plants. Cowpea, mung, urd, sunnhemp, gram, methi, kasuri methi and barseem infected with Pigeon pea mosaic virus [16] also showed reduction in number of nodules.

Sesbania infected with Sesbania mosaic virus, [5] mungbean infected with Common bean mosaic virus, [15] Soybean infected with Yellow mosaic, [10] Cowpea infected with Cowpea vein banding mosaic virus, Cowpea infected with Peanut stunt virus, [17] showed reduction in number of nodules due to virus infection. Fegla [6] Mali *et al.* [21] reported reduced nodulation in cowpea infected with Cowpea mosaic virus.

Reduction in number, size and fresh weight of nodules in the present study may be due to virus replication causing physiological alterations, [4] imbalance of auxin [20], and enzyme levels etc. [18] in the infected plants which directly or indirectly affect the symbiotic relationship of *Rhizobium* and cowpea plants. Tu *et al.* suggested that reduced nodulation in soybean infected with Soybean mosaic virus was probably caused by viral replication leading to physiological changes such as reduced photosynthesis, increased respiration and imbalance of auxin and enzyme levels. Van schreven [3] observed that shading of pea plants reduced nodule size and subsequent spray of sucrose improved its size and efficiency of N-fixation, but nodule number was not reduced by shading. Since CPGMV

infection reduced both size and number of nodules in cowpea the process is perhaps more complicated and not simple reduction of photosynthesis is the cause.

Since nodules of legumes are themselves auxin producers [11] and nodules of virus infected legumes could release growth regulating substances as in sweet clover infected with Wound tumor virus [1]. The reduction in nodule number of CPGMV virus infected cowpea plants observed under soil conditions might be related to an inhibition of auxin by combined nitrogen in the soil [9]

Rajagopalan and Raju [12] expressed the view that increase or decrease in nodule number might be associated with physiological status of roots and the density of the rhizosphere micro organisms; it might be possible that the changed physiological status of the root associated with low bacterial population in the soil has some role in reducing the nodulation in infected plants

The depression was probably due to virus replication causing physiological changes of reduced photosynthesis or increased respiration [8], imbalanced auxins and enzyme levels which directly or indirectly affected the rhizobium-cowpea symbiotic relationship. Ineffective symbioses are characterized by small nodules that fail to grow to normal size because the degeneration that starts in the bacterial region quickly spreads to the nodule meristem and stops its growth [14]. O'Hair and Miller [19] reported that cowpea strains of TMV were associated with a reduction in total nodules weight and nodules numbers. Dall et al. [2] showed nodulation losses of 31—67 % for three cultivars of barrel medic.

The effects of alfalfa mosaic virus on productivity of annual medic, annual burr medic, *Medicago polymorpha* showed decreased growth of AMV-infected plants, Although AMV infection resulted in no differences in the number of nodules formed in the first 11 d after germination but nodule mass was decreased by 23 % for virus-infected plants after 53 days and this difference disappeared after 75 days. Growth of AMV infected plants was decreased probably because of impaired N<sub>2</sub> fixation by nodules, so it seems that function of nodule is more affected rather than nodulation [7]. Nodulation was highly depressed by CABMV and CYMV in cowpea and it also affected the rhizobium-cowpea symbiotic relationship directly or indirectly [13]

### CONCLUSION

The cowpea plant infected with cowpea golden mosaic virus ( CPGMV ) showed significant loss in number, weight and volume of nodule in virus infected plants. Loss was more severe in early infected plants as compared to late infected plants. Although the weight and volume increases with the age of plant but that is still less than their healthy counterparts. Nodule number has not shown any significant increase after 40 days both in healthy and diseased plants. Volume of nodule was about 29 percent reduced over healthy and weight of nodule was reduced to 15 percent over healthy plants showing considerable loss due to virus infection on nodule formation.

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