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Effect of Cowpea Golden Mosaic Virus Infection on Chlorophyll Content in *Vigna Unguiculata* Leaf.

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ABSTRACT

Cowpea is an important legume crop and constitutes a vital food source of carbohydrate and proteins in many countries; Cowpea is prone to various diseases among which virus disease occupy an important place as they cause great loss in the yield. Present study deals with the effect of cowpea golden mosaic virus infection on chlorophyll content. Estimation of chlorophyll and carotenoid content was performed according to Arnon's method (1979), using specific absorption coefficient. Chlorophyll 'a' and chlorophyll 'b' increased with age in both healthy and diseased leaves. The chlorophylls were less in diseased leaves as compared to healthy ones. The averages were 13.24 mg for total chlorophyll 7.71 mg for chlorophyll 'a' and 5.63 for chlorophyll 'b' content for healthy leaves, whereas the values were 6.48 mg for total chlorophyll 3.83 mg chlorophyll 'a' and 2.76mg for chlorophyll 'b' in diseased leaves. carotenoids also increased in the leaves of healthy and diseased plants with increasing age the corotenoids were always less in diseased leaves compared to healthy ones.

Keywords: Cowpea, Geminivirus, Chlorophyll, Carotenoids.

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INTRODUCTION

Grain legumes are important constituents of protein in vegetarian diet in India. Among the grain legumes, cowpea is one of the important pulse crops. Cowpea is also cultivated for fodder, green manure, vegetable, and soil improving cover crop. In plains cowpea is cultivated in Kharif season, especially for grain and summer season for fodder.

Vigna unguiculata (L) walp is native of Central Africa since wild forms are found only there; Cowpea is now widely distributed throughout the tropics and subtropics. Of the total world production of over three million tonnes about 80% came from countries like of Central & West Africa and Nigeria [1] Cowpea is an important legume crop and constitutes a vital food source of carbohydrate and proteins in many countries. Crop is grown across the country major states are Karnataka, Kerala, Tamil Nadu, Madhya Pradesh and Uttar Pradesh,

Cowpea is one of the important pulse crops which are 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 [2] Present study deals with the effect of cowpea golden mosaic virus infection on chlorophyll content as the Chlorophyll, the green pigment of plants, is the most important component of the photosynthetic system. In plants, virus infection induce change in the coloration of leaves, a number of them showing mosaic or related symptoms, Mosaic causing viruses, though a diverse group, invariably affect the photosynthetic machinery of the host. Changes in the photosynthetic functions in leaves of Virus infection induces changes in host plant metabolic processes, including the most basic one, photosynthesis. Loss of photosynthetic activity, which is frequently reflected to macroscopic symptoms as yellow/green mosaic pattern or chlorosis of leaves, may be the result of decomposition processes or inhibited biosynthesis of some components [3]

METHODOLOGY

Estimation of chlorophyll and carotenoid content was performed according to Arnon's method [4] based on absorption of acetone extraction of chlorophyll at 663nm and 645nm, using specific absorption coefficient. The leaf material was extracted with 10ml of 80% acetone by macerating the tissue in a mortar and filtered through whatmann's filter paper No. 42 the residues left on the filter paper were again treated with 5ml of acetone several times with 60% and 40% acetone, till the filtrates contained no trace of green colour. The final volume of solution was made upto 50ml with 80% acetone. This was then centrifuged at 6000 r.p.m. for 20 minutes.

The optical density (O.D.) of the solution was measured in Carl Zeiss Jena specol-model-10 spectro colorimeter at 663nm and 645nm wavelength using 80% acetone as standard. The total chlorophyll content (chlorophyll a and chlorophyll b) values were determined by the following formulae.

Total Chlorophyll	=	$20.2\ O.D._{645} + 8.02\ O.D._{663}$ (mg/litre)
Chlorophyll a	=	$12.7\ O.D._{663} - 2.69\ O.D._{645}$ (mg/litre)
Chlorophyll b	=	$22.9\ D.D._{645} - 4.68\ O.D._{663}$ (mg/litre)

The chlorophyll content obtained in mg/liter was changed into mg/gm of fresh leaves.

The carotene content was estimated by the method of Ikan [5]. The alcohol extraction was done by the method described above, and the concentration of carotenoid was measured in mg/gm fresh weight by using the following formula.

Concentration of carotenoid = $A_o(440)/196$ x W mg/g f. wt.

where A_o = Optical density of Pigment solution of absorption at 440nm of spectrum.

W = gram of plant material/ml of final volume solution.

The observations are presented in Table 1 & 2

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RESULTS AND DISCUSSION

Results obtained in table 1&2 and Figure1-4 show that total chlorophyll content chlorophyll 'a' and chlorophyll 'b' increased with age in both healthy and diseased leaves. The chlorophylls were less in diseased leaves as compared to healthy ones. The averages were 13.24 mg for total chlorophyll 7.71 mg for chlorophyll 'a' and 5.63 for chlorophyll 'b' content for healthy leaves, whereas the values were 6.48 mg for total chlorophyll 3.83 mg chlorophyll 'a' and 2.76mg for chlorophyll 'b' in diseased leaves. It was observed that carotenoids also increased in the leaves of healthy and diseased plants with increasing age the corotenoids were always less in diseased leaves compared to healthy ones. Results of table 1&2 indicate that the Cowpea Golden Mosaic Virus infection has reduced the chlorophyll and carotenoid content of the infected plant. The colour in the leaves is due to a mixed effect of chlorophyll and carotenoids and their different proportions impart different shades of green, characteristic of the species. In the present study, a gradual change from green to yellow was observed in the leaves of virus infected cowpea. It was observed during the present study that the chlorophyll / carotenoid ratio in the leaves of healthy plant remains more or less unchanged whereas in virus infected plants it shows a steady decline it is, therefore, concluded that the mosaic symptoms in virus infected cowpea leaves are due to decreasing ratio of chlorophyll / carotenoids

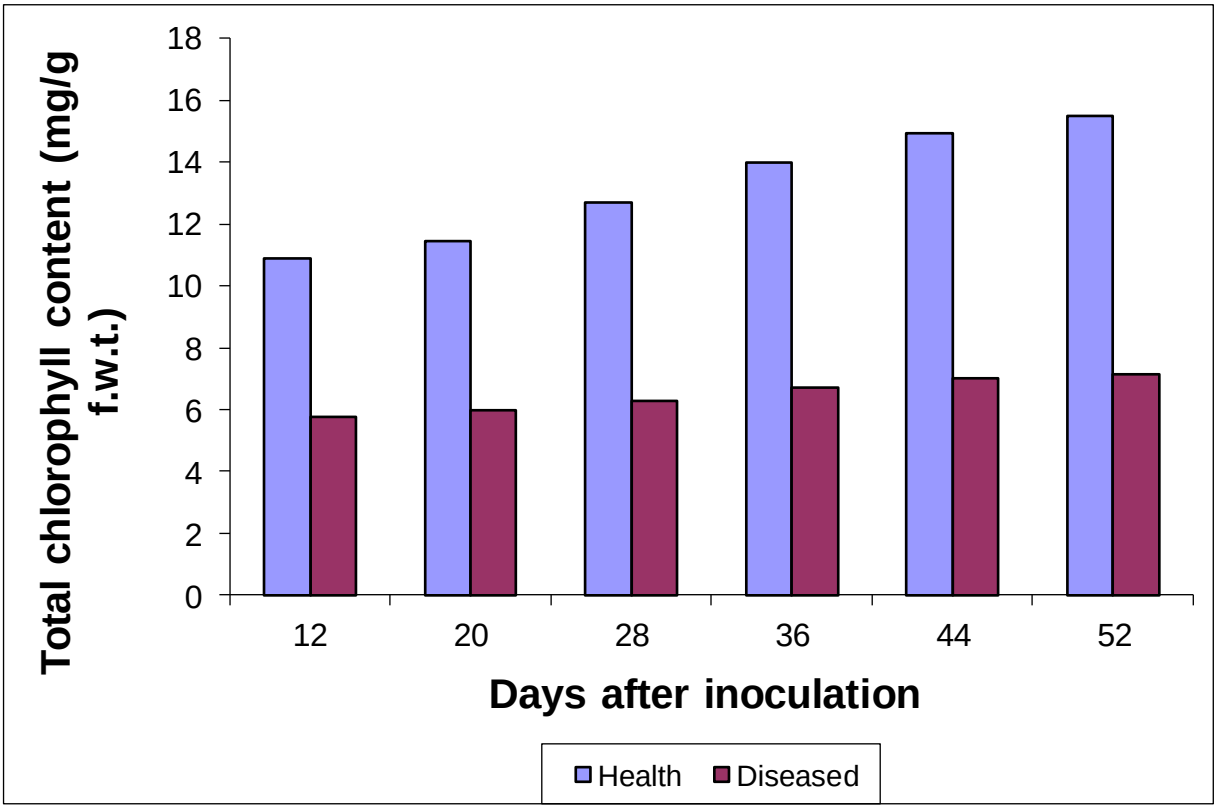

Figure 1: Effect of cowpea golden mosaic virus on infection on total chlorophyll content of *vigna unguiculata* leaf

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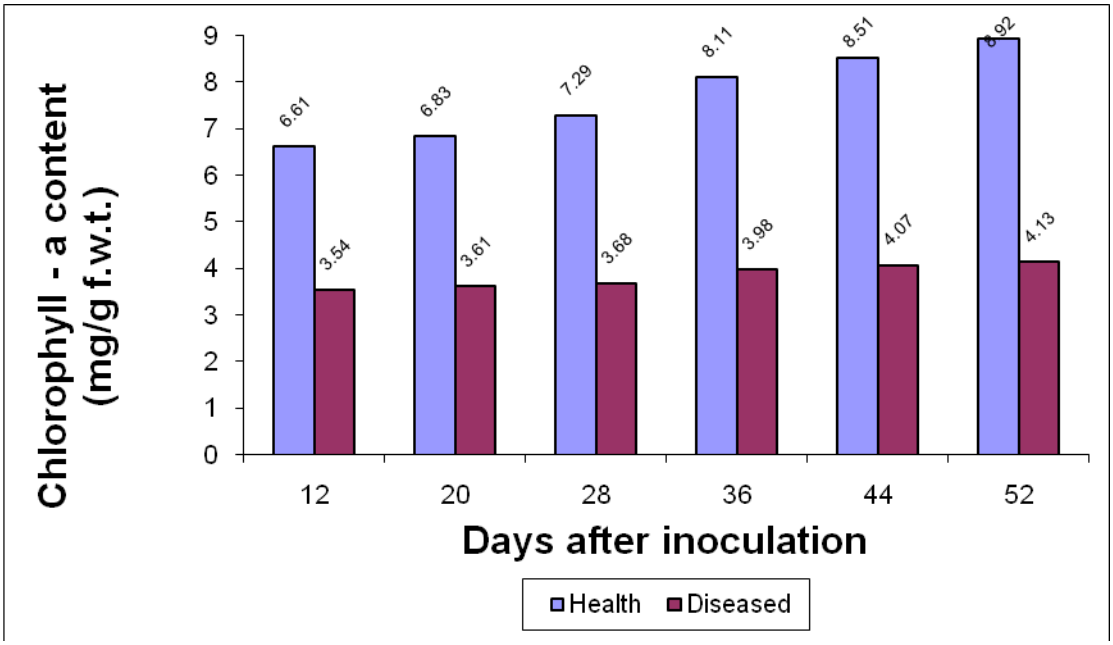

Figure 2: Effect of cowpea golden mosaic virus infection on chlorophyll a content of *Vigna unguiculata* leaf

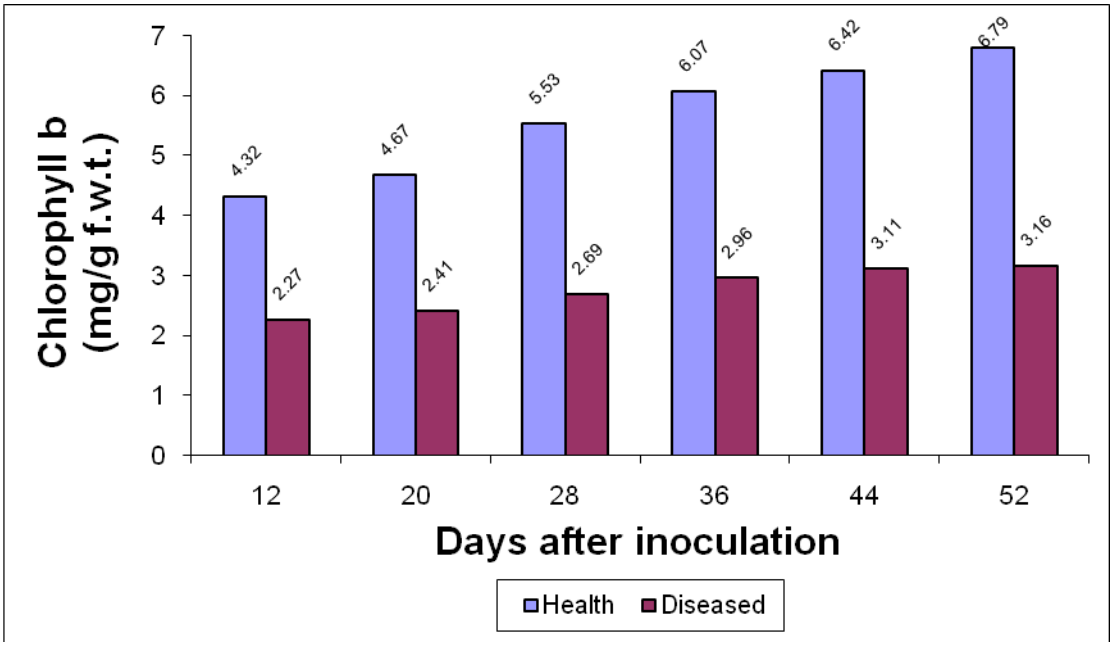

Figure 3: Effect of cowpea golden mosaic virus on chlorophyll-b content of *Vigna unguiculata* leaf

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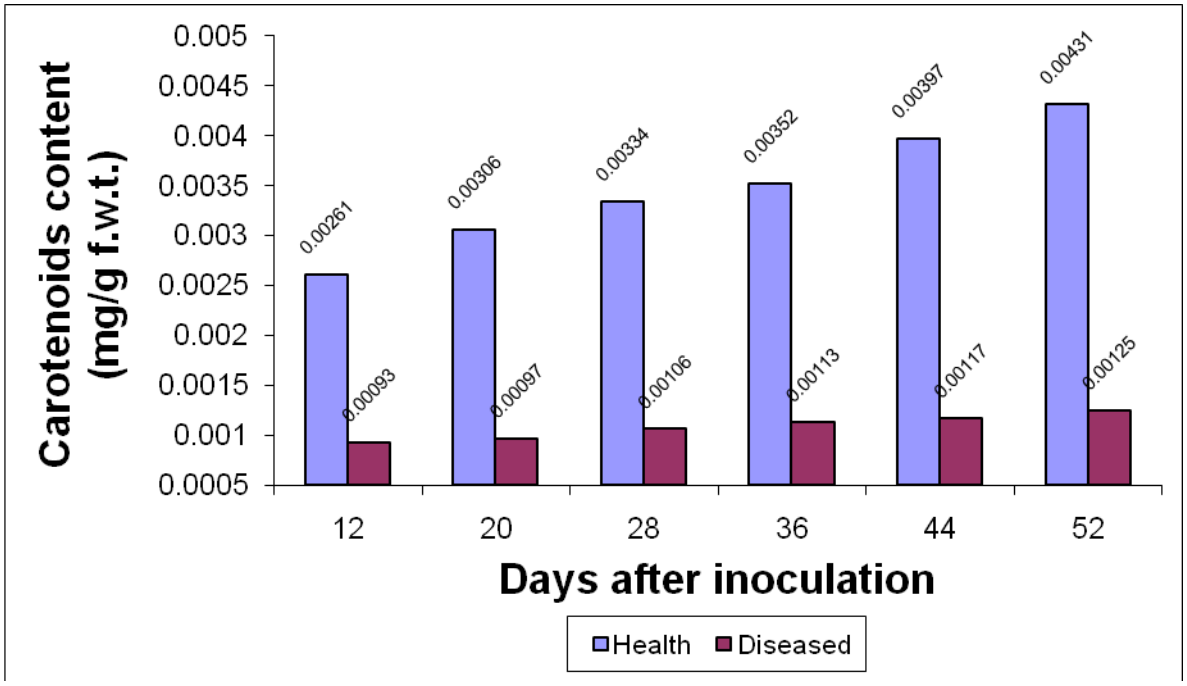

Figure 4: Effect of cowpea golden mosaic virus on infection on caratenoid content of *vigna unguiculata* leaf

Table 1: Change in Chlorophyll components in healthy and diseased leaf of *vigna unguiculata* infected with cowpea golden Mosaic virus

Days after inoculation	12	20	28	36	44	52
Total chlorophyll						
Healthy	10.87	11.46	12.71	13.99	14.92	15.51
Diseased	5.77	5.97	6.29	6.73	7.02	7.13
Chlorophyll-a						
Healthy	6.61	6.83	7.29	8.11	8.51	8.92
Diseased	3.54	3.61	3.68	3.98	4.03	4.13
Chlorophyll-b						
Healthy	4.32	4.67	5.53	6.07	6.42	6.79
Diseased	2.27	2.41	2.69	2.96	3.11	3.16
Carotenoids						
Healthy	0.00261	0.00306	0.00334	0.00352	0.00397	0.00431
Diseased	0.00093	0.00097	0.00106	0.00113	0.00117	0.00125

Table 2: Percentage change in Chlorophyll components in diseased leaf as compared to healthy leaf of *vigna unguiculata* infected with cowpea golden Mosaic virus

Days after inoculation	% decrease in total chlorophyll	% decrease in Chlorophyll a	% decrease in Chlorophyll b	% decrease in Carotenoids
12	51.0%	30.7%	20.5%	0.16%
20	54.9%	32.2%	22.6%	0.20%
28	64.2%	36.1%	28.4%	0.22%
36	72.6%	41.3%	31.1%	0.23%
44	79.0%	44.4%	33.1%	0.28%
52	83.8%	47.9%	36.3%	0.30%

Reduction in pigments have been reported in other host plants infected with different viruses by Yash and Chowfla, 1987; Suresh et al. 1988 and Ravinder et al. 1989, Mali et al 2000, Milavec etal 2001, Funayama-Noguchi & Terashima 2006, Pineda et al 2008, Singh & Shukla 2009.[6,7,8,9,10,11,12, &13].

Decrease in chlorophyll content is due to Chlorosis and necrosis of diseased plant parts. Meena et al observed chlorophyll content in healthy and diseased leaves of capsicum. Chlorophyll a, b and total chlorophyll content in healthy leaf of capsicum were 0.2834 mg/g, 0.1650 mg/g and 0.4932 mg/g of fresh weight of healthy leaf tissue, respectively, while in diseased leaf chlorophyll a, b and total chlorophyll content were decreased and reached up to 0.0489 mg/g, 0.0779 mg/g [14].

Several studies have focused on interactions between different plants and virus combinations. The level of total chlorophyll and carotenoids in control and Yellow vein mosaic virus infected leaves was studied by Palanisamy et al [15] who reported that total Chlorophyll and Carotenoids concentrations were significantly reduced in infected leaves by 64% and 62%, respectively. It was recorded that Zucchini yellow mosaic virus (ZYMV) infected leaves of pumpkin showed severe symptoms as mosaic, green blisters, size reduction and deformation and the virus infection diminished the Chlorophyll a (48%), Chl b (53%) and carotenoid contents (52%)

Similar observations were recorded in rice varieties by Emanual et al [16] but increase of chiorophyll content in Vigna mungo L. var. T 9 infected by urd bean leaf crinkle virus has been reported by Malik et al [17] . Marcos et al [18] described that Sunflower chlorotic mottle virus caused chlorotic mottling symptoms and important growth reduction and yield losses in sunflower. After symptoms became evident CO_2 fixation rate decreased, nevertheless soluble sugars and starch increased but chlorophyll contents decreased in infected leaves.

In the infected plants of Phaseolus vulgaris L. due to infection of bean golden mosaic virus Mali et al [9] found reduction in chlorophyll a and b in diseased plants of moth bean genotype infected with yellow mosaic virus. Bassanezi et al[19] observed reduction in chlorophyll content in leaves of Phaseolus vulgaris L. infected with bean line pattern mosaic virus. Lower chlorophyll content and higher carotenoid to chlorophyll ratio than those in intact and mock-inoculated controls, signs of senescence were observed in leaves with local lesions and in yellow leaves of infected plants [20]. Total chlorophyll, chlorophyll a, chlorophyll b content was lower in virus infected mungbean plant varieties and hyacinth bean [21,22].

The TMV infection slightly changed total chlorophyll, phenolic antioxidant compounds and soluble protein in infected papaya plant. The TMV infection leads to a decrease in chlorophyll a & b, total phenols and soluble protein by rate 47.89%, 7.89% and 61.35% in infected leaves. The total chlorophyll, chlorophyll a, chlorophyll b and carotenoid content were lower in infected tissue [13].

Chlorophyll, the green pigment of plants, is the most important component of the photosynthetic system. In plants, virus infection induces change in the colouration of leaves, a number of them showing mosaic or related symptoms. 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 a consequence of infection

Opinion differs regarding the change in levels of leaf pigment and expression of mosaic symptoms Smith [23] Bawden [24] and Matthews[25] favour the view that virus infection destroys the pigments in the chloroplast and thus causes a reduction in their level and hence the mosaic symptom. Cook [26] and Shaffield [27] on the contrary, are of opinion that the virus does not destroy the pigment in chloroplast but competes with plastids for some of the products of their synthesis and thus reduces the synthesis of the pigments which leads to mosaic symptom. [28]. The loss of chlorophyll in such cases has been attributed to the inhibition of the formation of new plastid units after virus infections rather than their destruction Diener, 1963, John 1963, Goodman et al. 1967, Ramakrishnan et al. 1969, Singh and Mall (1973, Singh and Srivastava 1979 and Sharma et al. 1980). Bianchini et al. (1998) reported reduced chlorophyll content [22]

Chinnadurai and Nair [29] claimed that chloroplast protein is utilized for the virus protein synthesis. Thus it seems probable that the virus competes with the chloroplast for the protein as suggested by Cook [30]. Since several proteins are involved in the formation of chloroplast skeleton, the diversion of the chloroplast

protein to virus protein will certainly affect the number and nature of the chloroplast and perhaps its efficiency also to bind with the photosynthetic pigments. This may be one of the possible reasons for the reduction in the pigment level in the leaves of virus infected plants.

REFERENCE

[1] Kochhar S. L. Economic Botany in the Tropics Macmillan Publisher 2012;,Edition : 4th ISBN-13 : 9789350590676
[2] Malathi, V.G., Usharani, J.S., Sivalingam, P.N., Rouhibaksh, A., Padma Latha, K.V. and Periasamy, M. Annu. Rev. Plant Pathal.2004; 3 : 225-270.
[3] Zoltán Szigeti, Asztéria Almási, Éva Sárvári, Proceedings of the 7 th Hungarian Congress on Plant Physiology, 2002 S03-05
[4] Arnon D I. Plant Physiology 1949; 24: 1-15
[5] Ikan I. Natural products: A laboratory guide. 1969, Academic Press,London.
[6] Yash Gupta and Chowfla, S.C. Indian J. Plant Pathal. 1987; 5 (I) : 46-48.
[7] Suresh, G.L., Rao, N.G. and Singh, B.G. South Indian Horticulture,1988; 36 (3) : 121-124.
[8] Ravinder, T., Rao, N.G. and Singh, B.G, Journal of Research APAV, 1989; 17 (I) : 70-72.
[9] Mali, P.C., Burman, Uday and Lodha, Satish (2000). Indian Phytopath. 2000; 53 (4) : 379-83
[10] Milavec, Maja, Ravnikar and Maja, Kovac .Plant Physiol. And Biochem.2001; 39 : 891-98.
[11] Funayama-Noguchi & Terashima Functional Plant Biol. 2006 33 : 165-175
[12] Pineda M. ,J. Soukupova, K. Matous, L. Nedbal & M. Baron Photosynthetica 2008,46: 441-451
[13] Singh, Vimla and Shukla, K. Ann. Plant Protec. Sci. 2009 17 : 1.
[14] Rishi Kesh Meena, Indu Singh Sankhla and Vidya Patni Plant Archives 2016;Vol. 16 No. 1, pp. 257-260 ISSN 0972-5210
[15] Palanisamy P, Michael P.I., Krishnaswamy M .Physiological and Molecular Plant Pathology 2009;74:129-133.
[16] Emanual N., S. Suresh and P. Ashok. Ann. Pl. Protec. Sci. 2002;10 : 212-215.
[17] Malik, Satish, Kumar, P., Panwar, J. D., Anjali, S.and Rathi, Y. P. S. Ann. Plant Protec. Sci. 2002;10 : 91-94.
[18] Marcos C., J. V. Gonçalves, G. Jurandi, M. Oliveira and M. A.Gomes. Fitopatol. Bras.2005; 30 : 128-136.
[19] Bassanezi, R. B., Amorim, L. Bergamin and Filho,A. Summa-Phytopathologica 2001;27 : 5-11.
[20] Mojca, Milavec, Maja, Ravnikar and Maja, Kovac. Plant Physiol. And Biochem.2001 39 : 891-98.
[21] Srivastava M. Gupta U. P. and A. sinha, J. Sci. Res. 2010; 54 : 135-152
[22] Manisha Srivastava, U.P. Gupta and Asha Sinha, Crop Res. 2012;44 (1 & 2): 174-181 [23]
[23] Smith, C.E. Science, 1924 9 : 155.
[24] Bawden, F.C. and Pirie, N.W. Ann. Rev. Pl. Physiol.,1952 3 : 177 - 188.
[25] Matthews, R.E.F.. Plant virology. Academic Press. Inc., 1991 San Diego
[26] Cook, M.T.. Dept. Agric. Purto Rico., 1931 15 : 117-181.
[27] Shaffield , F. N. L .Ann. Appl. Biol. (1933), 20 : 57-69.
[28] Harsányi A, Böddi B, Bóka K, Almási A, Gáborjányi R. Physiol Plant (2002) 114:149-155.
[29] Chinnadurai, G. and Nair, M.C.. Curr. sci., (1971). 40 : 18-19.
[30] Cook, M.T. Jour. Dept. Agric. Purto Rico., (1931). 15 : 117-181.