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Indexing of Yellow Vein Mosaic Disease of Mesta (*Hibiscus cannabinus*)

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Abstract: The assessment of severity with chronological development of disease of mesta (*Hibiscus cannabinus,* cv. HC-583) on a particular diseased plant was studied. Based on the symptomatology and intensity of diseases caused by *Begomovirus* an empirical formula was developed. A methodology for assessing the disease intensity of yellow vein mosaic disease of mesta grown under natural field condition was proposed and it was validated by comparing with infected plants grown under field as well as glasshouse conditions. Quantitative assessment of the disease indicated that the disease index value at different stages of pathogenesis varied from 1.82 to 49.44 under field condition whereas the same varied from 2.78 to 50.83 under glasshouse condition. Mortality of infected leaves was noticed when the index value reached maximum. With the present methodology scoring of gradual development of disease has become possible for a particular plant and even for an individual leaf of a particular cultivar.

Key words: Scoring formula, symptom, disease incidence, intensity

INTRODUCTION

The occurrence of yellow vein mosaic disease of mesta (*Hibiscus cannabinus* L; family Malvaceae) is a serious threat to disease scenario in India (Chatterjee and Ghosh, 2007a, b). It was found in endemic form in different parts of India during the last few years and cause great reduction in fibre and seed yield and thus a major threat to production (Chatterjee *et al*., 2005). The association of a novel *Begomovirus* (family: Geminiviridae) namely Mesta yellow vein mosaic virus with the disease was confirmed by electron microscopy and molecular techniques, sequence information and southern hybridization (Chatterjee and Ghosh, 2007a, b; Chatterjee *et al*., 2006). The disease is characterized by yellowing of veins and veinlets followed by complete chlorosis of the leaves of affected plants with the advancement of infection (Fig. 1). If the plants are infected by the virus at early stage of growth they do not flower even. The infected plants in general show stunted growth with reduced leaf size. This disease is transmissible by whitefly (*Bemisia tabaci*) under natural condition (Chatterjee *et al*., 2005). Now it is spreading at a faster rate at different parts of India like Andhra Pradesh, Uttar Pradesh, Bihar, Orissa and West Bengal and attracted immediate attention for in-depth study.

Many disease scoring techniques for assessing incidence and intensity caused by viruses have been reported in plants (Anonymous, 1976; Anjaneyulu, 1975; Furumoto and Mickey, 1967a, b; Joshi, 1973; Ghosh *et al*., 1981), but assessment of severity with chronological development of disease on a particular plant and even in a particular leaf of a diseased plant was never reported. In recent days these studies are neglected and for this purpose disease scoring was not attempted. It results into wrong information of disease intensity and disease incidence pattern. Moreover, for assessing intensity of diseases caused by viruses on fibre crops especially mesta has not yet been attempted. Beside this the typical yellow vein mosaic disease symptom caused by *Begomovirus* is unique and thus for chronological assessment of severity of the disease, development of an empirical formula was needed. Hence, the present investigation was an attempt to develop a methodology for assessing the disease intensity of Yellow Vein Mosaic (YVM) disease on mesta plants grown under field condition and to validate the method it was also compared with infected plants grown under field as well as glasshouse conditions.

MATERIALS AND METHODS

Inoculation of host and virus transmission at glasshouse condition: Thirty healthy mesta plants (*H. cannabinus* cv. HC-583), a highly susceptible cultivar to yellow vein mosaic (YVM) disease, were raised in earthen pots containing four seedlings per pot and filled with 4 kg of well-sieved field soil mixed with 1.5 g of urea in the glasshouse.

A virus-free stock of whitefly was reared on healthy tobacco plants (*Nicotiana tabacum*) in large wooden cages, covered with insect proof, galvanized wire mesh at

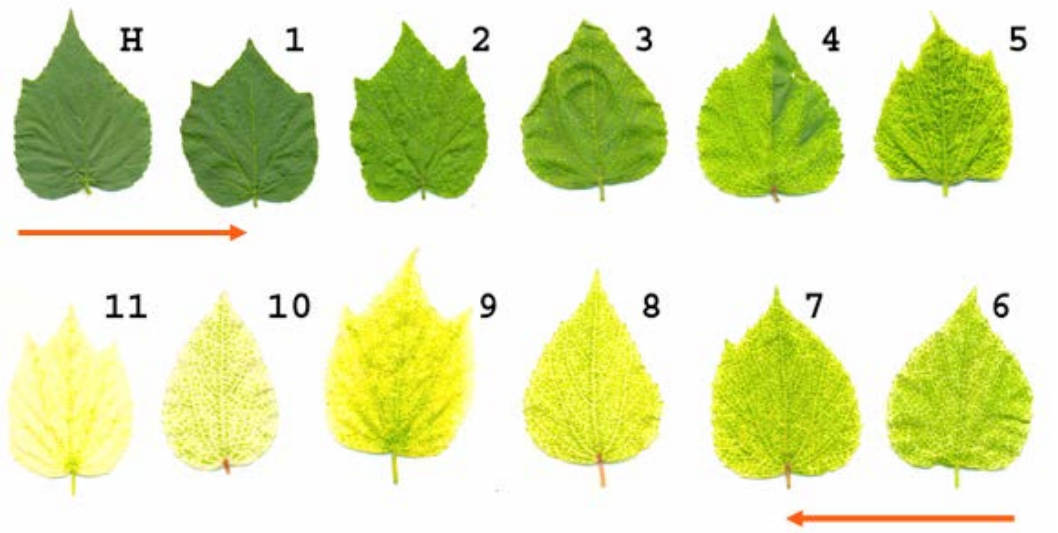

Fig. 1: Yellow vein mosaic disease development in *Hibiscus cannabinus* (cv. HC-583) plant; H: healthy leaf, 1-11: development of symptoms

30°C in a temperature-controlled glasshouse with a photoperiod of 18:6 h (L:D) and 60% RH. Adult whiteflies that emerged from nymphs were used in this study. Acquisition access of the virus-free whiteflies was given for 12 h on yellow vein mosaic infected mesta plants, maintained under glasshouse conditions. Twenty-day-old healthy plants at 6-leaf stage, maintained in glasshouse, caged individually with mylar polyester tubes (2" X 6") with two nylon screen side windows, were inoculated by confining 5 viruliferous insects in each cage for 12 h. Control plants were treated similarly with non-viruliferous insects. All plants were then kept in insect-proof temperature controlled glasshouse.

Disease transmission at field condition: Healthy seedlings of the same cultivar were grown 6 cm apart in small field plot (2×2.5 m) containing 100 plants in 3 replications maintained in randomized block design. The germinated plants were then allowed to grow under natural environment for disease development. After the appearance of first symptom the gradual development of the disease was noted at every 10 days interval in each plant and compared with those of grown and artificially infected with the virus under glasshouse condition for assessing the usefulness of the proposed scale.

Development of disease intensity index formula: Observations on plants grown under glasshouse condition were recorded at weekly intervals starting from 11 to 46 days of plant age after inoculation for successive four years and a disease intensity index formula was developed based on the symtomatology. Disease development was noted chronologically by considering different grades, *viz.* (A) development of foliar symptom, (B) nature of discoloration, (C) extent of yellowing of veins, (D) area of discoloration and (E) extent of stunting. Total gradation was based on a numerical value of 100 (Table 1). In the scale, each symptom syndrome was given equal importance and according to disease severity the symptom syndrome was divided into different gradations. Each grade was given a numerical value and noted with respect to each infected leaf (Table 1, 2). When the chlorotic spots started developing irregularly, the size of the individual area of discolorations was noted and their average was used to grade the area of discoloration (Table 1). In this way numerical values for each leaf of the individual plant were recorded separately and indexing was done according to the following formula:

$$\text{YVM disease intensity index} = \frac{[\sum_{s=A}^{D} L_{1s} + \sum_{s=A}^{D} L_{2s} + \sum_{s=A}^{D} L_{3s} + \ldots\ldots\ldots\ldots + \sum_{s=A}^{D} L_{ns}] + G}{T_N}$$

Where:

L_1, L_2, L_3, L_n = No. of affected leaves on a plant varying from 1 to n

s = Score of a diseased leaf varying from A to D

Lis = Total score on diseased leaf of a plant in a particular stage/ period (i = 1/2/3/…./n)

G = Growth habit of infected plant (i.e., extent of stunting = E)

T_N = Total number of leaves in a single infected plant

Table 1: Distribution of numerical values based on the importance of the factors in the proposed scale

Grade	Symptom syndromes	Numerical Scale for the severity	Maximum numerical value
(A)	Type (development) of foliar symptom (discoloration)		20
	No symptom	0	
	Appearance from one half	5	
	Appearance from both halves	10	
	Erratic appearance	15	
	Complete foliar discoloration	20	
(B)	Nature of discoloration		20
	No discoloration	0	
	Greenish yellowing	5	
	Yellow mosaic	10	
	Chlorotic yellowing	15	
	Complete chlorosis	20	
(C)	Extent of yellowing of veins		20
	No symptom	0	
	Appearance of small chlorotic flake	5	
	Coalescent of chlorotic flakes	10	
	Appearance of yellowing of veins	15	
	Appearance of yellow netting	20	
(D)	Area of discoloration		20
	Nil	0	
	25%	5	
	50%	10	
	75%	15	
	100%	20	
(E)	Extent of stunting (growth habit)		20
	Nil	0	
	Mild	5	
	Moderate	10	
	High	15	
	Severe	20	

Table 2a: YVM disease index at different stages of pathogenesis in cultivar HC-583 under field condition

Days after sowing	Total leaf No.	Total infected leaf	YVM infection value* (YVM index)
32	11	1	1.82±0.004
42	14	4	15.00±0.042
52	17	9	28.82±0.017
62	18	10	29.17±0.008
72	14	9	42.50±0.014
82	9	6	49.44±0.154

*Mean average of 100 plants±SE

Table 2b: YVM disease index at different stages of pathogenesis in cultivar HC-583 under glasshouse condition

Days after inoculation	Total leaf No.	Total infected leaf	YVM infection value* (YVM index)
11	9	1	2.78±0.018
18	14	6	18.93±0.034
25	16	8	26.25±0.205
32	16	8	30.63±0.158
39	16	10	37.19±0.090
46	12	10	50.83±0.061

*Mean average of 30 plants±SE

RESULTS

The present investigation revealed that under glasshouse condition the disease development on inoculated mesta (HC-583) plants was early as compared with those grown under natural field condition. But the disease symptoms and its chronological development under both the conditions appeared to be of similar nature. In a single plant not all the leaves had the symptoms due to infection. Symptom started in the emerging leaves in the form of scattered pin-head like light-yellow coloured irregular shaped spots, either one or both halves of the lamina including veins. The spots gradually increased in size by coalescing with each other leading to the yellow-chlorotic flecks on veins and yellow mosaic symptoms on inter-veinal regions. With the advancement of the disease, the infected lamina showed yellow chlorotic discoloration. Gradual yellowing of petiole and crown portion was also noticed in infected plants. In case of severe infection stunting of plant with defoliation of complete chlorotic leaves was observed. In addition to the above, the survival rate and life span of infected plants in glasshouse were also found less than those in field condition.

A successive four years epidemiological study on the YVM disease index at different stages of pathogenesis revealed that the infection severity varied from 1.82 to 49.44 in mesta (HC-583) under field condition, whereas under controlled glasshouse condition the same varied from 2.78 to 50.83 (Table 3a, b). Longer incubation period (nearly one month) for symptom expression was noticed under field condition, whereas under artificial inoculation in glasshouse the same observed to be around eleven days (Table 2a, b). Gradual development in severity of the disease leading to the index value of 49.44 and 50.83 at

Table 3a: Distribution of different numerical values and calculations of YVM disease index values using equation at different stages of pathogenesis under field condition (cultivar HC-583)*

Leaf position (top to bottom)	Days after sowing	Grade A	B	C	D	Extent of stunting (G = E)	Foliar score Individual $D \Sigma L_{is}$ s = A	Integrated n D $\Sigma \Sigma L_{is}$ I = 1 s = A	YVM disease intensity index n D[$\Sigma \Sigma L_{is}$] + G i= 1 s = A T_N
L_1	32	0	0	0	0	0	0		
L_2		0	0	0	0		0		
L_3		5	5	5	5		20		
L_4		0	0	0	0		0		
L_5		0	0	0	0		0		
L_6		0	0	0	0		0		
L_7		0	0	0	0		0		
L_8		0	0	0	0		0		
L_9		0	0	0	0		0		
L_{10}		0	0	0	0		0		
L_{11}		0	0	0	0		0	20	1.82
L_1	42	0	0	0	0	0	0		
L_2		10	10	5	20		45		
L_3		10	10	5	15		40		
L_4		0	0	0	0		0		
L_5		20	15	15	15		65		
L_6		15	15	10	20		60		
L_7		0	0	0	0		0		
L_8		0	0	0	0		0		
L_9		0	0	0	0		0		
L_{10}		0	0	0	0		0		
L_{11}		0	0	0	0		0		
L_{12}		0	0	0	0		0		
L_{13}		0	0	0	0		0		
L_{14}		0	0	0	0		0	210	15.00
L_1	52	10	5	5	15	5	35		
L_2		10	5	5	15		35		
L_3		10	10	10	5		35		
L_4		20	20	10	20		70		
L_5		20	20	15	20		75		
L_6		15	10	15	15		55		
L_7		10	10	10	20		50		
L_8		0	0	0	0		0		
L_9		20	15	15	15		65		
L_{10}		15	15	15	20		65		
L_{11}		0	0	0	0		0		
L_{12}		0	0	0	0		0		
L_{13}		0	0	0	0		0		
L_{14}		0	0	0	0		0		
L_{15}		0	0	0	0		0		
L_{16}		0	0	0	0		0		
L_{17}		0	0	0	0		0	485	28.82
L^1	62	10	5	5	10	10	30		
L^2		15	5	5	15		40		
L_3		10	10	10	20		50		
L_4		10	5	10	20		45		
L_5		10	10	10	15		45		
L_6	(Died)	-	-	-	-		-		
L_7	(Died)	-	-	-	-		-		
L_8		20	20	15	20		75		
L_9		10	20	15	20		65		
L_{10}		0	0	0	0		0		
L_{11}	(Died)	-	-	-	-		-		
L_{12}		15	15	15	20		65		
L_{13}		0	0	0	0		0		
L_{14}		0	0	0	0		0		
L_{15}		15	5	10	20		50		
L_{16}		0	0	0	0		0		
L_{17}		15	5	10	20		50		
L_{18}		0	0	0	0		0		
L_{29}		0	0	0	0		0		
L_{20}		0	0	0	0		0		
L_{21}		0	0	0	0		0	515	29.17

Table 3a: Contiuned

Leaf position (top to bottom)	Days after sowing	Grade A	B	C	D	Extent of stunting (G = E)	Foliar score Individual $D \Sigma L_{is}$ s=A	Integrated n D Σ Σ L_{is} i=1 s=A	YVM disease intensity index n D[Σ Σ L_{is}] + G i=1 s=A T_N
L_1	72	10	20	15	20	15	65		
L_2		15	20	15	20		70		
L_3		10	20	10	20		60		
L_4	(Died)	-	-	-	-		-		
L_5	(Died)	-	-	-	-		-		
L_6	(Died)	-	-	-	-		-		
L_7	(Died)	-	-	-	-		-		
L_8	(Died)	-	-	-	-		-		
L_9		20	20	15	20		75		
L_{10}	(Died)	-	-	-	-		-		
L_{11}	(Died)	-	-	-	-		-		
L_{12}		20	20	15	20		75		
L_{13}		0	0	0	0		0		
L_{14}	(Died)	-	-	-	-		0		
L_{15}		15	20	15	20		70		
L_{16}		0	0	0	0		0		
L_{17}		15	20	10	20		65		
L_{18}		15	5	10	20		50		
L_{29}		15	5	10	20		50		
L_{20}		0	0	0	0		0		
L_{21}		0	0	0	0		0		
L_{22}	(Died)	-	-	-	-		-		
L_{23}		0	0	0	0		0	580	42.5
L_1	82	20	20	15	20	15	75		
L_2	(Died)	-	-	-	-		-		
L_3		20	20	15	20		75		
L_4	(Died)	-	-	-	-		-		
L_5	(Died)	-	-	-	-		-		
L_6	(Died)	-	-	-	-		-		
L_7	(Died)	-	-	-	-		-		
L_8	(Died)	-	-	-	-		-		
L_9	(Died)	-	-	-	-		-		
L_{10}	(Died)	-	-	-	-		-		
L_{11}	(Died)	-	-	-	-		-		
L_{12}	(Died)	-	-	-	-		-		
L_{13}	(Died)	-	-	-	-		-		
L_{14}	(Died)	-	-	-	-		-		
L_{15}		20	20	15	20		75		
L_{16}		0	0	0	0		0		
L_{17}		15	20	10	20		65		
L_{18}		20	20	15	20		75		
L_{19}		15	20	10	20		65		
L_{20}		0	0	0	0		0		
L_{21}		0	0	0	0		0		
L_{22}	(Died)	-	-	-	-		-		
L_{23}	(Died)	-	-	-	-		-	430	49.44

*Mean average of 100 plants

field level and glasshouse condition, respectively, was noticed with the age of the plant. Mortality of the infected leaves was also noticed at the maximum infection level.

DISCUSSION

Present investigation highlighted the useful application of empirical formula developed for assessing disease intensity of yellow vein mosaic disease of mesta (HC-583). Ascribing of numerical values to some characters have been made in conducting the experiments and the sum total of those provided the index value on disease intensity. Variation in symptom expression in plants grown in glasshouse condition and under natural condition indicated that the lower index value obtained under field condition might be due to interaction of different epidemiological factors on plant growth and presence of lower population of the vector. Highest intensity or 100% infection was never noticed under natural and glasshouse condition and as a result YVM disease index value during this experiment never reached to 100%, as because all the infected plants died before it under both conditions. Hence, we stopped gradation at maximum value. Here, severity of 49.44 and 50.83 index

Table 3b: Distribution of different numerical values and calculations of YVM disease index values using equation at different stages of pathogenesis under glasshouse condition (cultivar HC-583)*

Leaf position (top to bottom)	Grade Days after inoculation	Foliar score A	B	C	D	Extent of stunting (G = E)	Individual $D \Sigma L_{is}$ s=A	Integrated n D $\Sigma \Sigma L_{is}$ i=1 s=A	YVM disease intensity index n D[$\Sigma \Sigma L_{is}$] + G i=1 s=A T_N
L_1	11	0	0	0	0	0	0		
L_2		0	0	0	0		0		
L_3		0	0	0	0		0		
L_4		5	5	5	10		25		
L_5		0	0	0	0		0		
L_6		0	0	0	0		0		
L_7		0	0	0	0		0		
L_8		0	0	0	0		0		
L_9		0	0	0	0		0	25	2.78
L_1	18	10	5	5	20	5	40		
L_2		10	5	10	10		35		
L_3		0	0	0	0		0		
L_4		15	15	15	20		65		
L_5		10	5	10	20		45		
L_6		0	0	0	0		0		
L_7		0	0	0	0		0		
L_8		0	0	0	0		0		
L_9		10	5	5	20		40		
L_{10}		10	5	5	15		35		
L_{11}		0	0	0	0		0		
L_{12}		0	0	0	0		0		
L_{13}		0	0	0	0		0		
L_{14}		0	0	0	0		0	260	18.93
L_1	25	0	0	0	0	10	0		
L_2		5	5	10	20		40		
L_3		10	10	10	20		50		
L_4		20	15	10	20		65		
L_5		0	0	0	0		0		
L_6		20	20	15	20		75		
L_7		20	15	10	20		65		
L_8		15	5	0	15		35		
L_9		0	0	0	0		0		
L_{10}		15	5	10	15		45		
L_{11}		10	5	0	20		35		
L_{12}		0	0	0	0		0		
L_{13}		0	0	0	0		0		
L_{14}		0	0	0	0		0		
L_{15}		0	0	0	0		0		
L_{16}		0	0	0	0		0	410	26.25
L_1	32	0	0	0	0	10	0		
L_2		0	0	0	0		0		
L_3		5	10	10	20		45		
L_4		20	20	10	20		70		
L_5		20	20	15	20		75		
L_6		0	0	0	0		0		
L_7	(Died)	-	-	-	-		-		
L_8		20	20	15	20		75		
L_9		20	10	10	20		60		
L_{10}		15	10	10	15		50		
L_{11}		0	0	0	0		0		
L_{12}		15	10	10	15		50		
L_{13}		15	10	10	20		55		
L_{14}		0	0	0	0		0		
L_{15}		0	0	0	0		0		
L_{16}		0	0	0	0		0		
L_{17}		0	0	0	0		0	480	30.63
L_1	39	10	5	10	10	15	35		
L_2		5	10	10	20		45		
L_3		20	10	10	20		60		
L_4		20	20	15	20		75		
L_5		20	20	15	20		75		
L_6		0	0	0	0		0		

Table 3b: Contiune

Leaf position (top to bottom)	Grade Days after inoculation	Foliar score A	B	C	D	Extent of stunting (G = E)	Individual $D \Sigma L_{is}$ s=A	Integrated n D $\Sigma \Sigma L_{is}$ i=1 s=A	YVM disease intensity index n D[$\Sigma \Sigma L_{is}$] + G i=1 s=A T_N
L_7	(Died)	-	-	-	-		-		
L_8	(Died)	-	-	-	-		-		
L_9		20	20	15	20		75		
L_{10}		15	15	15	20		65		
L_{11}		0	0	0	0		0		
L_{12}		20	15	10	15		60		
L_{13}		15	10	10	20		55		
L_{14}		10	5	5	15		35		
L_{15}		0	0	0	0		0		
L_{16}		0	0	0	0		0		
L_{17}		0	0	0	0		0		
L_{18}		0	0	0	0		0	580	37.19
L_1	46	5	10	10	20	15	45		
L_2		10	10	10	20		50		
L_3		10	15	15	20		60		
L_4		20	20	15	20		75		
L_5	(Died)	-	-	-	-		-		
L_6	(Died)	-	-	-	-		-		
L_7		0	0	0	0		0		
L_8	(Died)	-	-	-	-		-		
L_9	(Died)	-	-	-	-		-		
L_{10}		10	10	10	20		50		
L_{11}		5	5	5	15		30		
L_{12}	(Died)	-	-	-	-		-		
L_{13}		20	20	15	20		75		
L_{14}		0	0	0	0		0		
L_{15}		20	20	15	20		75		
L_{16}		20	10	10	20		60		
L_{17}		20	20	15	20		75		
L_{18}	(Died)	-	-	-	-		-		
L_{19}	(Died)	-	-	-	-		-		
L_{20}	(Died)	-	-	-	-		-		
L_{21}	(Died)	-	-	-	-		-	595	50.83

*Mean average of 30 plants

value was sufficient enough to consider the whole plant totally infected at field level and glasshouse condition, respectively. As by 100% infection, quantifications is not clearly emphasized, so the gradation of either 49.44 or 50.83 infection value was clearer for maximum infectivity, in a particular plant as evident by the validation of the formula in fields as well as glasshouse condition.

The present investigation showed that in a single plant not all the leaves had yellow mosaic discolorations and even if discolorations were present, they were not necessarily of the same type because cells vary in their susceptibility. This type of symptom development was also noticed in previous study on another diseases (Furumoto and Mickey, 1967a, b). Furthermore, susceptibility of one leaf differs from another and the infected areas also differ between leaves as was observed by early studies (Kado, 1972). Hence, gradual development of the disease and the cumulative effect of all factors are the most important criteria to designate a single plant about its severity of infection. Consideration of symptom expression as determined by visual observation may lead to incorrect assessment of severity (Joshi, 1973). This present experiment took account of all such possible factors associated with YVM disease incidence and gradual disease development in a particular plant and even in the individual leaf. So, it is not improbable to conclude that the empirical formula developed during the present investigation may be helpful for assessing severity of YVM disease on mesta.

CONCLUSION

The present study is an attempt towards the understanding of chronological disease development of mesta yellow vein mosaic caused by a *Begomovirus*. It is a unique approach to contribution as because such type of basic study is rarely attempted now-a-days, though the experts of plant pathology and agriculture are regularly shouting in this direction to attract the researchers mind. Without having a correct disease scoring technique, which is applicable in all situations, we can not be in position to

declare the disease intensity and incidence on universal level. Moreover, it will resist to claim a disease an epidemics. From the corner of disease management and information to farmers the disease indexing also has immense importance.

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