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Survey of Begomovirus affecting cucurbits in Bihar

Ranveer Kumar, Srinivasaraghavan A and Jaya Bharti

Abstract
A total of 39 locations were surveyed to know the status of begomovirus like symptoms in different cucurbits growing area of Bihar. The locations were selected in 13 districts in such a way that it covers all the agroclimatic zone of Bihar. The observed disease severity and incidence were found to be moderate to severe at different locations. The average disease incidence was found to be maximum in Banka (60.55%) followed by Kishanganj (59.60%). The lowest average disease incidence was found under Nawada (41.50%) followed by Nalanda (43.20%), similarly the maximum disease severity was of 42.30 per cent was recorded in Nawada with disease severity followed by of Madhubani (38.75%). Whereas the average minimum disease severity percent was found in Nalanda (24.20%) followed by Gopalganj (25.50%) and Vaishali (25.70%). Among these districts, the highest average incidence was found under Zone II (59.05%) followed by Zone IIIA (57.28%) and Zone I (51.16%) and least was at Zone IIIB (49.74%) likewise across different zones of Bihar, the maximum average disease severity was seen under Zone IIIA (36.06%) followed by Zone I (32.38%) and minimum was under Zone IIIB with 29.56 per cent severity. All the isolate further processed and cloned with the degenerative primer of begomovirus and found to be positive to the CP Deng and specific ToLCNDV.

Keywords: Begomovirus, locations, agroclimatic, disease severity, disease incidence

1. Introduction
Cucurbit under Cucurbitaceae family considered as economically important vegetable grown throughout year with global production of 151million tons. Cucurbits are considered as a key source of carbohydrates, vitamins and minerals (Nath, 1994) [20]. India produces about 5.47 million tons of Cucurbits including pumpkin, squash, gourd, cucumber and melons over an estimated area of 5.64 lakh ha which is nearly share 75 percent production of south Asia (Rai *et al.*, 2008) [22]. There are 825 species under 118 genera of Cucurbitaceae out of which 37 genera with 100 species have been reported in India. Cucurbits are cultivated round the year in Indo-Gangetic plains of Bihar which gives major supply of vegetables in the region. The Indo-Gangetic plains especially diara part of Bihar and other eastern Indian states has immense potential of growing the cucurbits and shares a major part of cucurbit production in India (Kumari *et al.*, 2018). The family Cucurbitaceae is one of the largest groups which includes members *viz*., "Pumpkin (*Cucurbita moschata* Duch. Ex Poir), Ash gourd (*Benincasa hispida* Thunb.), Ridge gourd (*Luffa acutangula* (L.) Roxb.), Bitter gourd (*Momordica charantia* L.), Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.), Cucumber (*Cucumis sativus* L.), Gherkin (*Cucumis anguria* L.), Sponge gourd (*Luffa cylindrica* L.) Roem.), Snake guard (*Trichosanthes cucumerina* L.), Ivy gourd (*Coccinia cordifolia* L.), pointed gourd (*Trichosanthes dioica* L.), Musk melon (*Cucumis melo* L.), Water melon (*Citrullus vulgaris* Schrad.), Summer squash (*Cucurbita pepo* L.) and winter squash (*Cucurbita maxima* Duch.)" (Seshadri, 1996) [25]. Among all the constraints of cucurbits production, the losses caused by the diseases incited by viruses pose greater threat due to the involvement of insect vector, difficulty in diagnosis and unavailability of proper management option (Mansour and Almusa, 1982) [16]. About 59 different viruses have been reported on different cucurbitaceous crops, among them begomoviruses belonging to the family Geminiviridae are considered as an important threat causing low cucurbit production (Tiwari *et al.*, 2010; Phaneendra *et al.*, 2012) [29, 21]. Initially the disease was known to occur in central-western India as pumpkin yellow vein mosaic disease. But later the virus was found to occur in different cucurbits *viz*., bitter gourd, cucumber, muskmelon, sponge gourd and winter squash *etc*. (Raj and Singh, 1996; Varma and Giri, 1998; Singh *et al.*, 2001; Khan *et al.*, 2002) [20, 30, 27, 13]. In addition to that, the epidemics of leaf curl in muskmelon yellow vein mosaic in pumpkin occurred during 1990s in Northern parts of India was an important event which brought attention towards begomoviruses in cucurbits (Varma and Malathi, 2003) [31].

The epidemics of 1990s was later attributed to the rapid spread of whitefly population in the early season cucurbits (Legg *et al.*, 1994) [15]. The members of the family begomovirus incite wide range of symptoms in cucurbits starting from, mild to severe mosaics, mottling, veins clearing, yellowing, leaf curling, puckering, leaf distortions, stunting *etc*. ultimately resulting in yield and quality reduction (Muniyappa *et al.*, 2003; Sharma *et al.*, 2012) [17, 26]. Until the beginning of the 21st century only two species of begomoviruses were reported to be infecting cucurbits in India *viz*., *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) and *Squash Leaf Curl China Virus* (SLCCNV) (Singh *et al.*, 2001; Sohrab *et al.*, 2003) [27, 28]. The association of *Indian Cassava Mosaic Virus* (ICMV) in yellowing and puckering symptoms of bitter gourd (Rajinimala and Rabindran, 2007) [24] and *Tomato Leaf Curl Palampur Virus* (ToLCPMV) along with *Pepper Leaf Curl Betasatellite* (*PepLCB*) was found associated with yellow mosaic disease of pumpkin (Namrata *et al.*, 2010) [19]. The members of the genus Begomovirus are either typically bipartite (New world begomoviruses) or either mono and bipartite (Old world begomoviruses) spread across the globe. The genome of a typical bipartite begomovirus is made up of two circular DNA molecules with an approximate size of 2.6-2.7 kb each, designated as DNA-A and DNA-B (Briddon *et al.*, 2001; Mansoor *et al.*, 2003) [6, 17], whereas, monopartite begomoviruses contain single circular DNA component known as DNA A. Both mono and bipartite begomoviruses are known be associated with small circular DNA molecules known as alpha and Beta satellites (Harrison *et al.*, 2002) [10]. The current efforts towards management of begomovirus mainly include development of resistant cultivars and management of vector through various means. The emerging reports of increasing host range of the virus and revelation of several weed species as reservoirs of the virus indicates the high level of variability among the begomovirus population. In spite of intensive efforts to manage the whitefly transmitted viruses, the epidemics incited by emerging or re-emerging Begomoviruses are quite frequent and appearing in previously unreported geographical locations. The molecular studies to reveal the species complex diversity has substantially contributed towards the understanding of evolution and ecology of begomoviruses and their epidemiological implications (Varma and Malathi, 2003) [31]. The major reasons attributed for high variability among the begomovirus population is involvement of recombination and pseudo-recombination due to mixed infection which can pose a serious threat to sustainable agriculture, particularly in the tropics and subtropics (Kirthi *et al.*, 2002) [12]. According to Dasgupta *et al.* (2003) [7], the extent of yield loss caused by some Geminiviruses is as high as 100 per cent. Several Begomoviruses have been reported to infect in different cucurbits across the globe (Al-Musa *et al.* 2011) [2]. The increasing incidence of whitefly transmitted begomoviruses is alarming and there is a need to develop a comprehensive strategy for their management. Understanding the population structure including the extent of spread and severity of virus species involved, diversity within and among the population of different begomovirus species is of paramount importance towards design and deployment of effective management strategy. Keeping these points in mind, a systematic study was conducted with following objectives to document the begomovirus infecting cucurbits and its distribution all over Bihar.

2. Methods and Materials

2.1 Survey and sample collection

A roving survey was conducted during summer and kharif season of year 2017-2018 in different cucurbit growing area of all the sub agroclimatic zone of the Bihar to record the disease incidence and severity of Begomoviruses in eastern part of India. Randomly five fields were selected at particular location and within them three spots of $5m^2$ were randomly identified and average disease incidence were calculated by counting total number of plants and number of plants showing distinct disease symptoms. Disease severity was also recorded based on the level of diseased plants and amount of plant parts affected. At each location information's like, crop, age of the crop, characteristic symptom along with particulars of the location including GPS data was recorded. The GPS data was recorded with help of location finder application of android based smartphone (Model: Xiomi Note 5 Pro). Details of location where survey conducted and diseased sample were collected are given below (Table 1 & Figure 1). In each field sample were collected from mild to severe infected symptomatic plants where four to five leaves express distinct symptoms of curling, mottling, crinkling, mosaic, yellowing, vein clearing, puckering etc. A total of 39 sample were collected from different locations across Bihar. Among 39 samples 20.51%sample were collected from Zone -I, 7.69% from Zone-II and 30.76%, 41.02% respectively from the Zone III A&B. After collections, the samples were kept in separate plastic or envelop to avoid mixing and detail of sample were written on the envelop and in register for future reference.

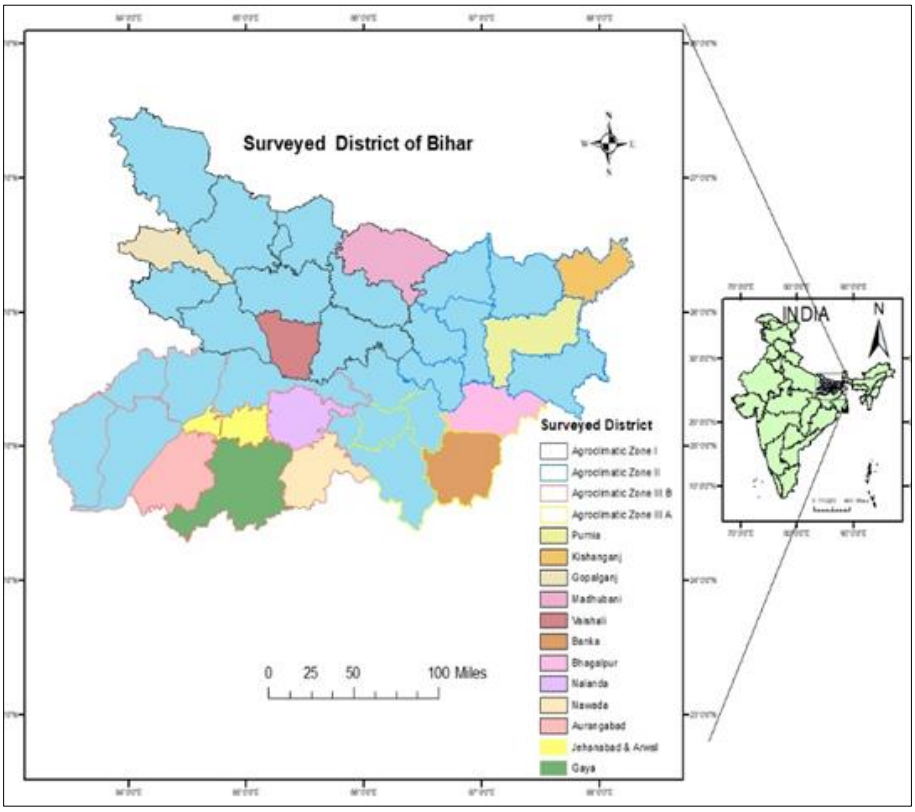

Fig 1: Location Map of Surveyed District of Bihar

Table 1: List of Location surveyed for begomovirus infected cucurbits field

	S. No	District	Location
Bihar Zone III (B)	1	Jehanabad	Karhara, Bandhu Ganj, Damuhan, Makhdumpur, Jehanabad
	2	Gaya	Chakand, Belaganj, Khaneta, Tekari
	3	Arwal	Kurtha, Karpi
	4	Nalanda	Bihar sharif, Nur sarai, Ekangarsarai
	5	Nawada	Hisua, Nawada
Bihar Zone HI (A)	6	Bhagalpur	Sabour, Naugachhia, Sarmaspur, Babupur, Vikramshila
	7	Banka	Banka
Bihar (Zone H)	8	Kishanganj	Arrabari
	9	Purnea	BPSAC
Bihar (Zone I)	10	Gopalganj	Basdila Khas, Bheriya
	11	Madhubani	Pariharpur, Fatehpsr
	12	Darbhanga	Yakubpur, Basdeopur
	13	Vaishali	Dih Buchauli, Husaina Khurd

2.1.1Calculation of Disease incidence and Disease severity

For each field five $5m^2$ area was randomly selected for calculating disease incidence and disease severity. Total No. of diseased plants and the total no. of plant were counted and disease incidence was calculated using the formula given below.

$$D.I(\%) = \frac{\text{Number of plants showing symptoms}}{\text{Total number of plants in a row}} \times 100$$

Disease severity was calculated by observing the percent leaf area showing symptoms in the infected in leaves. Leaves from five randomly selected plants were observed and Average Disease Severity were calculated using the following formula.

$$D.S(\%) = \frac{\text{Amount of leaf area having symptoms}}{\text{Total leaf area}} \times 100$$

2.2 Molecular detection and confirmation of Begomovirus

The DNA was isolated from genomic DNA isolation Kit ("Wizard Genomic DNA Purification Kit"; Make: Promega Bioscience). The standard procedure for DNA extraction was followed as per manufacturers instruction (Anon, 2019). After extraction of DNA with the help of Promega DNA purification kit, the quality of the DNA was assessed through gel electrophoresis system (GeNei bio-systems) at 1 per cent agarose gel in TAE buffer (1%).

2.2.1 PCR for Amplification with degenerative primers: Polymerase chain reaction was carried out in PCR machine (Eppendorf Master Cycle) with different annealing temperature for different primer up to 40 cycle of denaturation, annealing and extension. List of primers used are given in (Table 2.) and composition of reaction prepared for PCR given below in (Table 3). PCR product analysis with the gel electrophoresis using 1 per cent agarose gel with the 1 kb ladder. The electrophoresis was run for 30 minutes at 50V. Gel were checked under the Gel documentation system (GeNei).

Table 2: List of primers which were use specific primer for Begomoviruses

Oligo-nucleotides	Sequence 5' to 3'	Annealing temperature (^{0}C)	Reference
ToLCNDV-CPF	TACGATCTTGFCCG.AG.ATCTCA	53	Primers were deigned Iran the DNA-A of ToLCNDV (INE807949)
ToLCNDV-CPR	ACCCAGGTCCTTAAGTACCT		
ToLCNJV-sbo F	GAAATTAMTCG.AAGCGACC	50	Anssr *et al*, 2018
ToLCNJV-sbo R	TCTTAAGGGTGITTAGGACC		
Cp Deng 541 F	TAATATTACCKGMCGBCCSC	54	Deng *et al*. (1994)
Cp Deng 540 R	TGGACYTIRCkWGGI3CCITCACA		
MYMIV CPF	ATGGATTCCGGTGCATGTTG	50	Nimuctetin *et al.*, 2011
MYMIV CPR	G.ACITCTGGG.ATGATCTTATCGA		
Beta 01	GGTACCACTACGCTACGCAGCAGCC	50	Ridden *et al.* (2002)
Beta 02	GGTACCTACCCTCCCAGGGGTACAC		

Table 3: Material used for preparation of PCR reaction mixture of 25µL

Master mix	12.5µL
Nuclease free hater	8.5µL
Forward primer	1µL
Reverse primer	1µL
DNA Template	2µL

3. Result

3.1 Survey

Roving survey was conducted in different district of Bihar to know the current status of occurrence, distribution and severity of the begomoviruses in cucurbitaceous crops. Thirteen districts of Bihar (Jehanabad, Gaya, Arwal, Nalanda, Nawada, Bhagalpur, Banka, Purnea, Kishanganj, Gopalganj, Mahbubani, Vaishali and Darbhanga) were selected under Zone III A, Zone III B, zone II and Zone-I (figure 2). A total of 39 location were surveyed. The observed disease severity and incidence were found to be moderate to severe at different locations. The details of the survey are given below under different headings. At each location the typical symptoms were recorded and samples for molecular detection were also collected. Isolate were coded alphanumerically on the basis State, District, crop name and numerical value to avoid Mixing of Isolates.

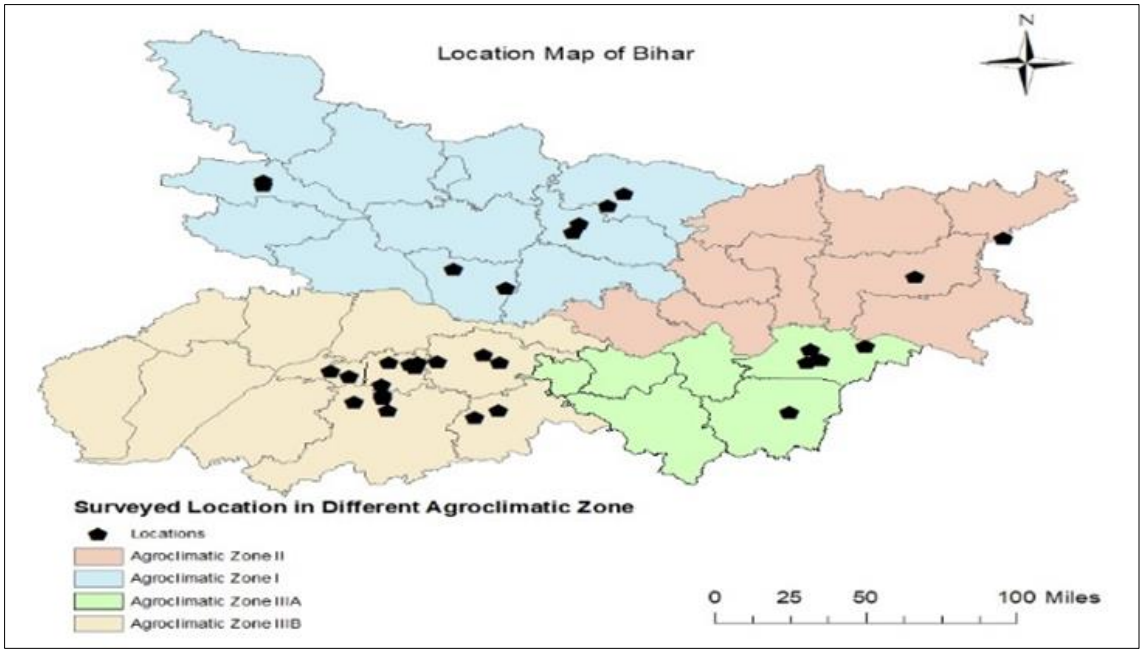

Fig 3: All 39-locations map of Coordinates Covering all the Agroclimatic Zone of Bihar

3.1.1 Disease Incidence

Disease incidence observed at different locations signify the presence of population of begomovirus at different places surveyed during 2018. The data presented in table 3. reveal that; among different surveyed districts of Bihar, the average disease incidence was found to be maximum in Banka (60.55%) having range between 56.90 to 64.20 per cent followed by Kishanganj (59.60%) where incidence ranged between 56.90 to 64.20 per cent. The lowest average disease incidence was found under Nawada (41.50%) with range of 38 to 45 per cent followed of Nalanda (43.20%) where, DI ranged from 40.00 to 46.50 per cent and in Gopalganj district the average disease incidence was 46.80 per cent which varied between 53.40 to 40.20 per cent. The average incidence in other seven districts was found to be 50.56,51.3,52.45,54, 54.1,54.85,55.45, per cent under Jehanabad, Darbhanga, Vaishali, Bhagalpur, Madhubani, Gaya and Arwal respectively. Among these districts the highest average incidence was found under Zone II (59.05%) followed by Zone IIIA (57.28%) and Zone I (51.16%) and least was at Zone IIIB (49.74%). Among all the 39-location surveyed, the highest disease incidence was found at Vikramshila in district Bhagalpur and the least disease incidence per cent was recorded at Basdila Khas in district Gopalganj (40.2%). Across all the locations and different cucurbits, it has been observed, maximum per cent disease incidence was found in

Pumpkin at Vikramshila (Bhagalpur) with 66.5 per cent followed by bottle gourd of Arrabari (Kishanganj) with 65.6 per cent and 64.30 per cent in Bitter gourd at Belaganj. The least disease incidence 40.20 per cent was found in Cucumber at Basdila Khas in district Gopalganj.

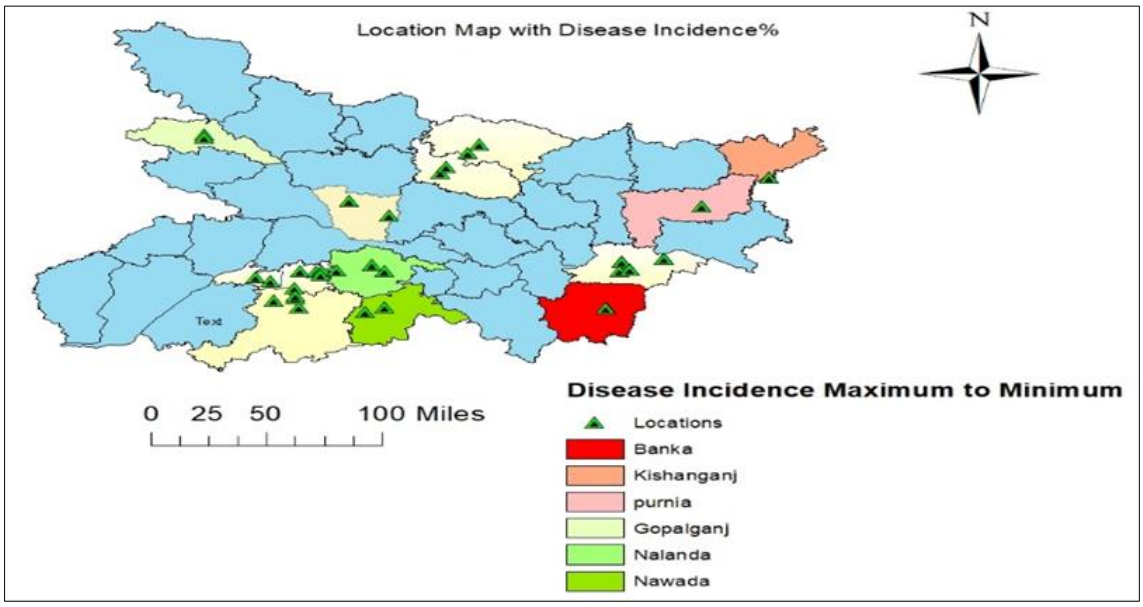

Fig 4: Map Representing Variability in the Disease Incidence%

3.1.2 Disease severity

At all the 39 locations surveyed, apart from disease incidence, disease severity (%) was recorded to understand the extent of begomovirus infection in the infected field. The data presented in (Figure 4 &Figure 5) indicates, a moderate level of severity was recorded based on the amount of plant parts showing the suspected symptoms. Across different zones of Bihar, the maximum average disease severity was seen under Zone IIIA (36.06%) followed by Zone I (32.38%) and minimum was under Zone IIIB with 29.56 per cent severity (Figure. 6). Among all the 13 districts surveyed from Bihar, the maximum disease severity was of 42.30 per cent was recorded in Nawada with disease severity ranging from 36.27 to 48.20 per cent followed by of Madhubani (38.75%) having range between 38.3 to 39.20 per cent. Whereas, disease severity in Bhagalpur (36.27%) having range between 29.3 to 43.6 per cent. The average minimum disease severity percent was found in Nalanda (24.20%) followed by Gopalganj (25.50%) and Vaishali (25.70%). The disease severity in Jehanabad, Purnea, Gaya, Arwal, Kishanganj, Darbhanga and Banka were 26.16, 29.8, 29.92, 32.6, 33.5, 34.75 and 35.85 per cent respectively (figure 3. & 4). Among all the above 39 locations the maximum disease severity per cent was present at Nawada in bottle gourd crop with 48 per cent severity, followed by cucumber at Bhagalpur and sponge gourd at Banka having 43.60 and 43.30 per cent severity. Similarly, the least disease severity was observed in the infected snake gourd field of Jehanabad (19.40%) followed by cucumber field at Ekangar of Nalanda (21.40%) and bottle gourd field at Bihar Sharif, Nalanda (21.60%).

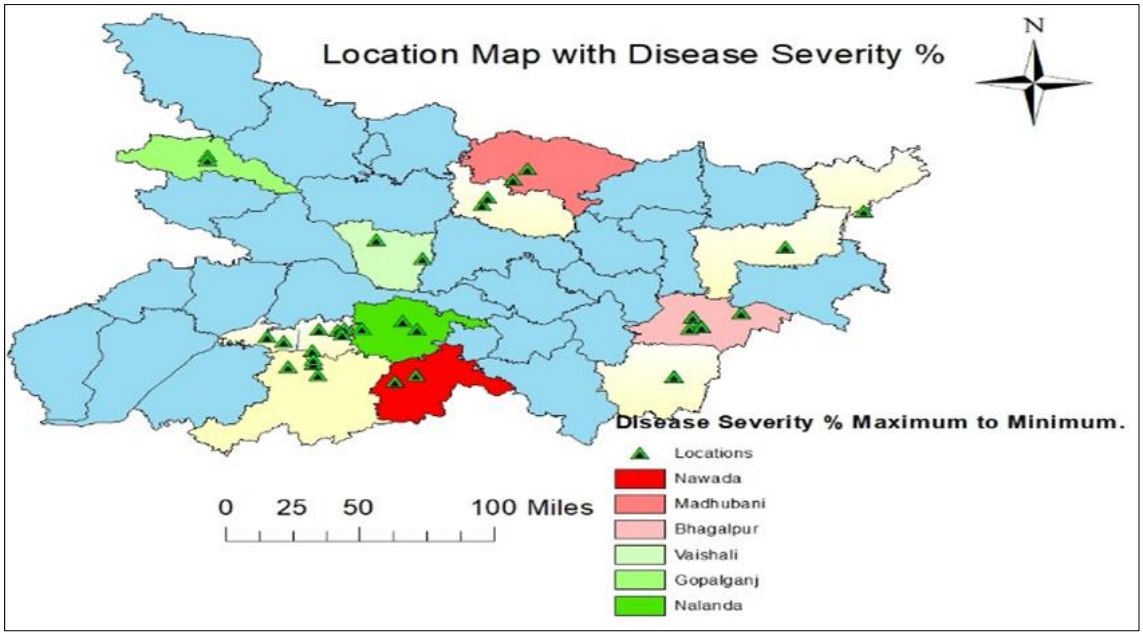

Fig 5: Location Map of Bihar with variable Disease Severity%

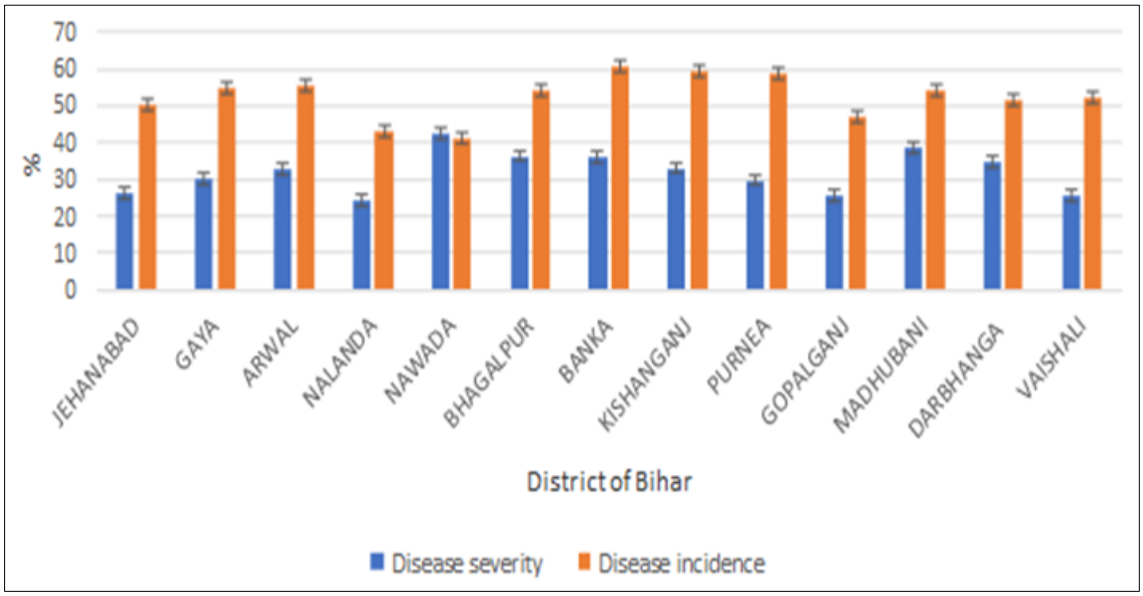

Fig 6: Disease incidence and Disease severity of the cucurbits showing begomovirus like symptoms.

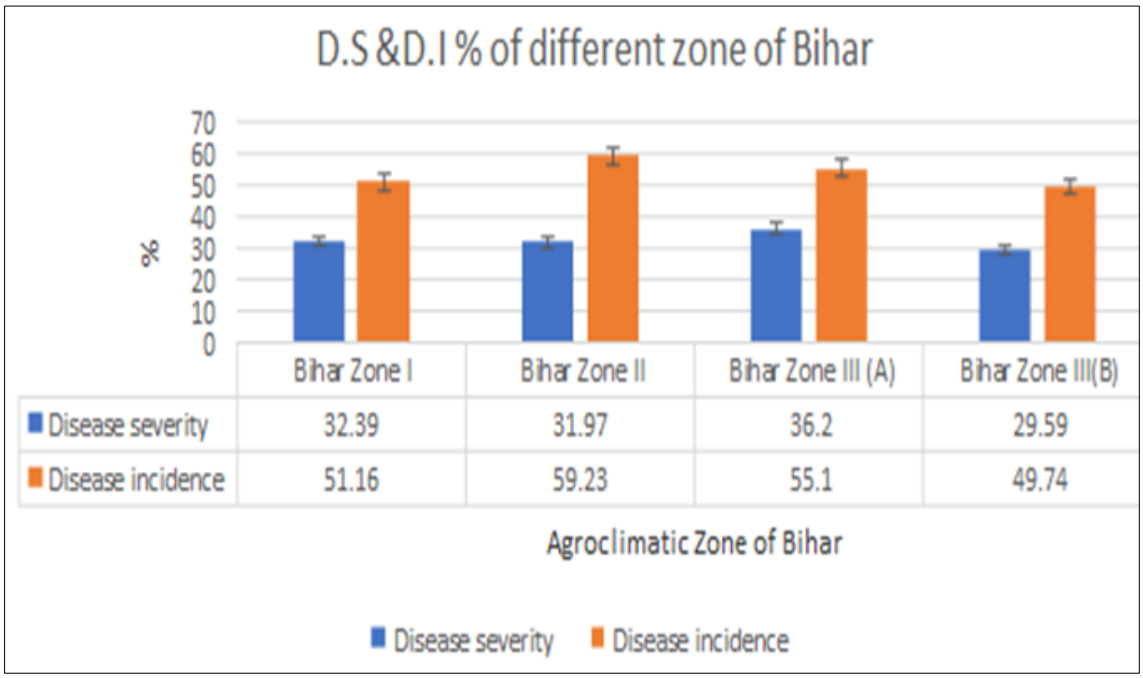

Fig 7: Graph representing the Disease severity and Disease Incidence on cucurbits showing Begomovirus like symptoms

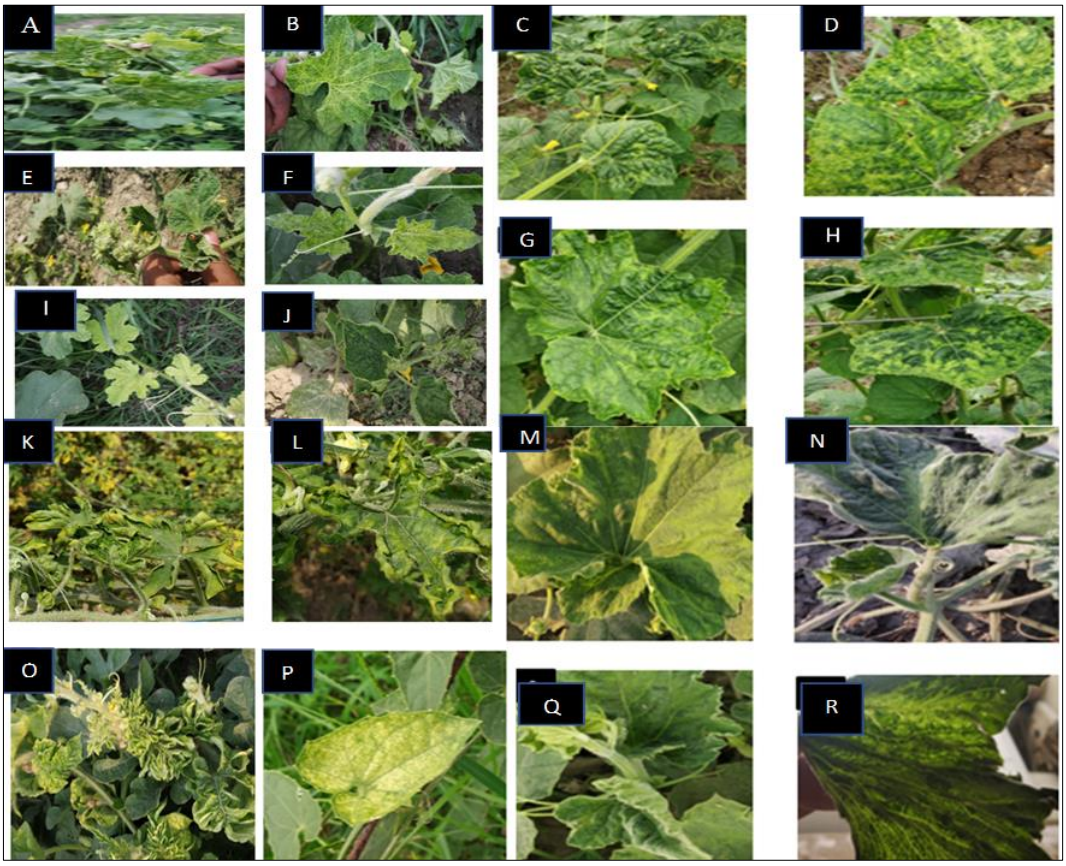

Fig 8: Variability of begomovirus symptoms observed on cucurbits. On Muskmelon (A) russeting of leaf (B) yellowing and vein clearing (E) Leaf curl (F) Mosaic Vein clearing (I) Yellowing (J) cupping of leaves. On Cucumber (C) Leaf curl (D) Yellow Mosaic (G) Mild Mottling (H) Puckering. On Bitter gourd (K) crinkling of leaves (L) Curling of leaves. On Bottle gourd (M) Mild mottling (N) Mild Mosaic (Q) Leaf curl(R) Vein clearing. On Watermelon (O) russeting of leaves, puckering and mottling. On Pointed gourd (Q) yellowing and mosaic (R) leaf curling

3.2 Symptomatology

All the survey was conducted during the flowering stage and post flowering stage of the crops. Different viral symptoms were observed at different cucurbits crops and at different locations an array of symptoms was recorded during the survey. The conspicuous symptoms observed were yellowing, mild to severe mosaic, mottling, curling, vein clearing, vein banding, puckering, crinkling, rosetting, leaf distortion, stunting etc. The symptomatic variability of begomovirus infection has been represented in different photographs taken during survey in (figure 8).

3.3 Detection of begomoviruses from symptomatic cucurbit plants

A total of 39 symptomatic leaf samples of cucurbits collected from different locations were subjected to molecular detection of begomovirus using degenerative primers (Deng). All the 39 isolates originating from cucurbits *viz*., bottle gourd (13), cucumber (9) water melon (1), sponge gourd (7), pumpkin (1), pointed gourd (2), musk melon (1), bitter gourd (3) and snake gourd (2) exhibiting various symptoms including puckering, curling, rosette, mild to severe mosaic, mild to severe mottling, distortion of leaves, cupping, etc. gave positive amplification. The positive amplification of approximate 570bp band indicated the involvement of one or more begomoviruses across locations with variety of symptoms.

4. Discussion

Cucurbits occupy an important position in every day diet offering rich amount of carbohydrates, vitamins and minerals. The Cucurbitaceae family is one largest plant family having lot of its members being cultivated across the world in a commercial scale (Nath, 1994) [20]. The crop is cultivated widely across the country including eastern India, Indo-Gangetic plains of Bihar especially diara lands shares a major part of cucurbit production in India (Kumari *et al.*, 2018) [14]. The fact that, increasing reports of cucurbit being affected by viral diseases especially whitefly transmitted begomoviruses across the country (Phaneendra *et al.*, 2012) [21] and lack of availability of a systematic study indicating the status of begomoviruses infecting cucurbits in eastern India especially in Bihar has inspired to undertake the current task of deducing the status of begomoviruses of cucurbits in Eastern India. The result of the present study has been discussed critically under this chapter.

4.1 Occurrence and distribution of begomoviruses in Bihar

A total of 39 location of Bihar were covered under survey to record disease incidence and severity of begomovirus like symptoms on cucurbits. Among all location surveyed covering three states, the highest average disease incidence found to be at district Banka with 60.55 per cent and the least disease incidence percent was found at the Nawada (41.50). The highest average incidence was found under Zone II (59.23) followed by Zone IIIA (57.28) and Zone I (51.16). Whereas the least disease incidence was found in Zone IIIB with 49.74 per cent. Across all the location and different cucurbits, it has been observed that maximum percent disease incidence was found in bottle gourd. Across all the locations, the maximum disease severity percent was present at Nawada

in Bottle gourd crop with 48.2 percent severity, followed by cucumber at Bhagalpur and Sponge gourd at Banka having with 43.60 and 43.30 percent severity. Similarly, the least disease severity was observed in the infected snake gourd field of Jehanabad (19.40%) followed by cucumber field at Ekangar of Nalanda (21.40%) and Bottle gourd field at Bihar Sharif, Nalanda (21.60%). Survey results clearly indicate that apparent symptoms of begomoviruses have been observed across all the surveyed locations with moderate to severe incidence as well as severity. The incidence of the disease at all the location was observed to be higher as compared to severity indication the recent and continuing infection. The available literature on occurrence of Begomoviruses on cucurbits also indicate the emerging nature of the virus on cucurbits with increasing host range (Dasgupta *et al.*, 2003; Varma and Malathi, 2003; Borah and Dasgupta, 2012; Jyothsna *et al.*, 2013; Nagendra *et al.*, 2014) [7, 31, 5, 11, 18].

4.2 Symptomatic diversity of begomoviruses infecting cucurbits in Bihar

The symptoms of begomoviruses recorded during the survey across three states of eastern India revealed an array of symptoms across the location and crops. A huge amount of variability was revealed within and among the crops and locations. The conspicuous symptoms observed were yellowing, mild to severe mosaic, mottling, curling, vein clearing, puckering, crinkling, rosetting, leaf distortion, stunting, cupping etc. in mild severe form. Vasudeva and Samraj (1948) for the first time have symptoms mosaics, mottling, leaf curl, vein clearing, reduced leaf size, stunting, puckering, deformation of leaf and crumping in cucurbits. These symptoms were later attributed to the whitefly transmitted viruses by various studies. Muniyappa *et al.* (2003) [17] observed that distinctive symptoms *viz*., blistering, cupping along with yellow mosaic of leaves in pumpkin plants arterially inoculated with yellow mosaic disease. Similar symptoms have also been reported on sponge gourd (Sohrab *et al.*, 2003) [28], bitter gourd (Rajinimala and Rabindran, 2007) [24] and Bottle gourd (Sohrab *et al.*, 2010) [28]. Symptoms *viz*., chlorosis, stunting, curling and crumpling of foliage was seen on watermelon infected by Cucurbit leaf crumple virus (*CuLCrV*) (Hagen *et al.*, 2008) [9]. Abhishek and Kang (2009) [1] reported symptoms like yellow vein, vein clearing and general yellowing in pumpkins and Snap melons infected with Begomoviruses. Were severely stunted and exhibited symptoms like chlorotic spots and mild leaf curl when infected with Chlorotic curly stunt disease. The variability of symptoms was not limited to any particular location and crop. The most common symptoms like, mosaic and mottling were observed across all location and all cucurbits. Whereas, rosetting, leaf curl and cupping were restricted only to few location where severe incidence and severity was noted.

5. Conclusion

A total of 39 location of Bihar were surveyed for to unearth the status of begomovirus like symptoms on cucurbits. Across all the location and different cucurbits, it has been observed that maximum percent disease incidence was found in bottle gourd. Maximum disease severity was present at Nawada in Bottle gourd crop with 48 percent severity, followed by cucumber at Bhagalpur and Sponge gourd at Banka having with 43.60 and 43.30 percent severity. Survey results clearly indicates that, apparent symptoms of begomoviruses have been observed across all the surveyed location with moderate to severe incidence as well as severity. The incidence of the disease at all the location was observed to be higher as compared to severity indicating the recent and continuing infection. A huge amount of symptomatic variability was revealed within and among the crops and locations. The conspicuous symptoms observed were yellowing, mild to severe mosaic, mottling, curling, vein clearing, puckering, crinkling, rosetting, leaf distortion, stunting, cupping etc. in mild severe form. The variability of symptoms was not limited to any particular location and crop. The most common symptoms like mosaic and mottling were observed across all location and all cucurbits. Whereas, rosetting, leaf curl and cupping was restricted only to few location where severe incidence and severity was noted. All the 39 isolates originating from cucurbits *viz*., bottle gourd (13), cucumber (9) water melon (1), sponge gourd (7), pumpkin (1), pointed gourd (2), musk melon (1), bitter gourd (3) and snake gourd (2) exhibiting various symptoms were found positive for presence of begomovirus. Out of 39 isolates tested all showed positive to the Begomovirus Deng primer out of which 37 was positive with the specific ToLCNDV specific primer. So at the end of the chapter, we can conclude with that cucurbits were found invariably having begomovirus like symptoms across all the surveyed locations The incidence and severity of the begomovirus across eastern India was found to be moderate to severe The incidence levels were high as compared to actual severity of the symptoms during the survey when crop was under flowering and post flowering stage. This indicates that there is temporal progress of the incidence.

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