



## A comprehensive survey on the occurrence of begomovirus infecting major cucurbits in Odisha State, India

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**ABSTRACT:** This study presents a comprehensive assessment of begomovirus disease incidence and severity in major cucurbits of Odisha from 2021 to 2023. A total of 320 samples were collected across various cucurbit crops, revealing diverse virus symptoms such as mosaic patterns, blistering, leaf curling and chlorosis. Disease incidences ranged widely, with bitter gourd, bottle gourd, ridge gourd, sponge gourd, cucumber, pumpkin, snake gourd, and ash gourd exhibiting varying percentages. Noteworthy disease incidence percentages (66-87%) were documented in different locations, providing a detailed insight into the regional impact of virus infections. The PCR-based detection using begomovirus-specific degenerate primers identified the begomovirus in 68 bitter gourds, 26 bottle gourds, 59 cucumber, 67 pumpkin, 66 ridge gourd, 11 sponge gourd, 8 snake gourd, and 9 ash gourd samples. Among these, 64.42% samples were infected with bipartite begomovirus, 60.74% samples with monopartite begomovirus and 12% samples with betasatellites. The infection rates varied across different locations, indicating the spatial dynamics of disease prevalence. These findings contribute valuable insights into the disease severity of cucurbit viruses in Odisha, offering a foundation for future research and management strategies in cucurbits cultivation.

**Keywords:** Cucurbit, begomovirus, disease incidence, PCR-based detection

### INTRODUCTION

The Cucurbitaceae family, comprising approximately 125 genera and 965 species, stands as the major family of fruits and vegetables globally. Widely cultivated in India and other tropical regions, these climbing or trailing vines with tendrils have been utilized for culinary purposes since ancient times (Chomicki *et al.*, 2019). Cucurbitaceous plants, encompassing fruits, seeds, stems, and leaves, hold significance not only in gastronomy but also in traditional medicine, particularly within the realms of Ayurveda and folk practices (Salehi *et al.*, 2019). India's vast cultivation landscape includes numerous cucurbit varieties, contributing significantly to both commercial and kitchen gardening sectors. The Food and Agriculture Organization (FAO) estimates that cucurbits cover about 5.6% of India's total vegetable production, spanning an extensive 4,290,000 hectares with a productivity of 10.52 t/ha (FAOSTAT, 2021).

In the state of Odisha, cucurbits find a prevalent place in subsistence farming, particularly in semi-urban and rural areas, meeting the escalating demand

for vegetables in urban regions. This cultivation has not only emerged as a profitable venture for resource-poor farmers, contributing to household income but has also created ongoing employment opportunities for field workers. Major cucurbit species in this agroecological landscape include cucumber, pumpkin, ash gourd, musk melon, snake gourd, bottle gourd, bitter gourd, ridge gourd, watermelon, and summer squash (Tripathy *et al.*, 2014). Despite their economic significance, cucurbit crops face formidable challenges, particularly from whitefly-transmitted begomoviruses of Geminiviridae family. Begomoviruses, with circular single-stranded DNAs, exhibit bipartite (DNA-A and DNA-B) as well as monopartite (DNA A) genomes and are associated with satellite DNA, such as alphasatellite and betasatellite (Neoh *et al.*, 2023). "In India, the predominant vectors of begomoviruses are whiteflies (*Bemisia tabaci*), which transmit the viruses in a circulative, non-propagative manner. Till date 24 begomovirus species were reported to be infecting cucurbits worldwide. Among them, tomato leaf curl New Delhi virus (ToLCNDV), squash leaf curl China Virus (SLCCNV) and tomato leaf curl

Palampur virus (ToLCPaV) are the major begomoviruses in Indian subcontinent inducing mosaic diseases in cucurbits, characterized by symptoms like leaf curling, mosaic mottling, crinkling, rosetting, reduced leaf size, yellow vein distortion, mosaic patches, and stunted growth (Kumari *et al.*, 2022). These diseases can result in significant yield losses, with reported incidences of up to 100% and losses exceeding 60% (Jeger, 2020).

Extensive research on begomovirus has been conducted in various states, including Tamil Nadu, Uttar Pradesh, and Chhattisgarh, revealing high infection rates (Nagendran *et al.*, 2017; Kumari *et al.*, 2021; Sahu *et al.*, 2021). However, Odisha's specific ecological zones demand a dedicated investigation to comprehend the distribution of begomoviruses associated with Cucurbitaceous crops. The present study, conducted between 2021 and 2023, aims to fill this gap by examining the occurrence and level of incidence of viruses infecting cucurbit crops across the diverse agro-climatic zones of Odisha state, India. This research seeks to contribute valuable insights into the challenges faced by cucurbit cultivation in the region, addressing the need for real-time solutions to combat viral diseases and mitigate economic losses in this vital agricultural sector.

## MATERIALS and METHODS

### Survey and virus sample collection

A comprehensive survey of major cucurbit crops was conducted across fifteen districts of Odisha state during the cropping seasons from 2021 to 2023. Leaves exhibiting characteristic virus symptoms were collected from various essential cucurbit crops, including bitter melon, ash gourd, ridge gourd, snake melon, cucumber, bottle melon, sponge melon, and pumpkin. Each site included three separate fields, and data on virus symptoms and disease incidences were documented (Krishnan *et al.*, 2023). Disease incidence data was determined by calculating the ratio of virus-infected plants to the total number of plants in the field (Ranveer Kumar *et al.*, 2021). In total, 320 samples were collected in separate bags to prevent cross-contamination, and these diseased leaf samples were transported to the laboratory for further analysis.

### DNA Extraction

The leaf samples were subjected to total genomic DNA extraction followed the CTAB method (Aboul-Maaty and Oraby, 2019). A 100 mg of symptomatic leaf

samples were weighed and ground into a fine powder using liquid nitrogen. The powdered sample was then mixed with CTAB isolation buffer (3ml of 10% CTAB, 2.8ml of 5M NaCl, 0.4ml of 0.5M EDTA, 1ml of 1M TrisHCl, 0.3g of PVP for 10ml of CTAB buffer), and 30µl β-mercapto -ethanol. After incubation at 65°C, chloroform isoamyl alcohol (24:1) was added, and the supernatant obtained after centrifugation was processed with isopropanol and sodium acetate. The resulting DNA pellet was flow over with ethanol, air-dried, and reconstituted in TE buffer after RNase treatment.

### DNA Concentration

The concentration of DNA was spectrophotometrically measured at 260 nm (A<sub>260</sub>) using Nano spectrophotometer (Nano Drop, Nabi, Korea). The absorbance ratios at A<sub>260</sub>/A<sub>280</sub> and A<sub>260</sub>/A<sub>230</sub> were estimated to assess DNA purity concerning protein and polysaccharide contamination. Additionally, the extracted DNA was assessed through electrophoresis on a 0.8% agarose gel stained with ethidium bromide.

### PCR Assay based detection

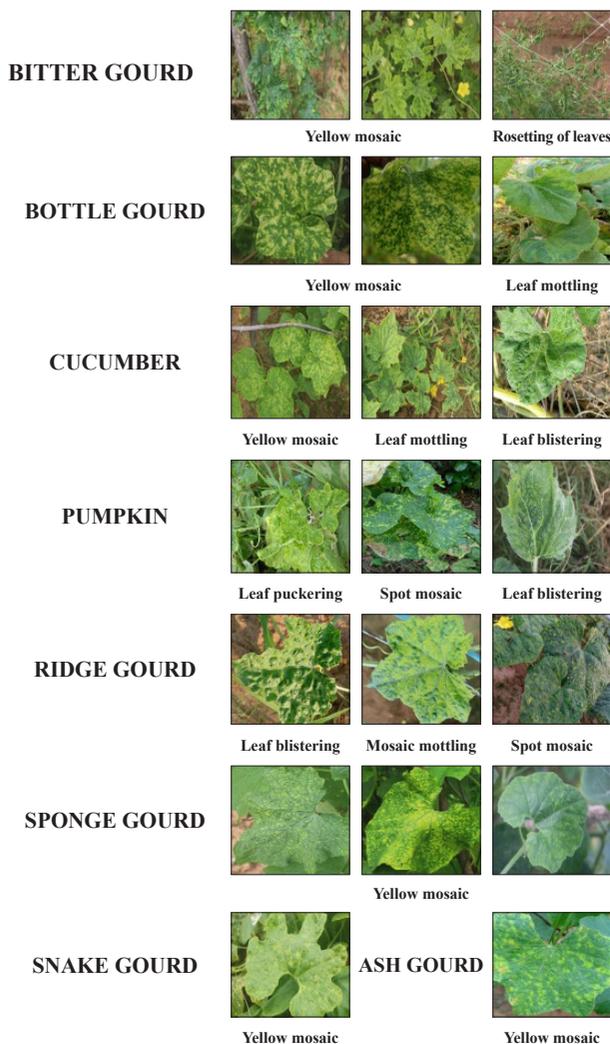
For begomovirus detection, universal degenerate primers and genus-specific primers targeting the DNA-A and DNA-B component, and abutting primer pair of betasatellite were employed in the PCR assays mentioned in the Table 1. The 2 × Emerald AMP GT PCR Master Mix (Takara, Japan) was used for the amplification in the PCR. The PCR reaction was performed in the 25µl volume with the mixture consisting of 12.5µl of 2 × master mix, 2.5µl each primers (10nmol), 2.5µl of template DNA (100ng) and the final volume make up with nuclease-free water. The resulting PCR products were analyzed by gel electrophoresis on a 2% agarose gel with the PCR conditions as mentioned in the Table 1.

## RESULTS AND DISCUSSION

Diversity in cucurbits enthralling with substantial economic value, are widely popular in Indian subcontinent. However, occurrence of begomovirus infections in a large way is posing a substantial threat to cucurbit production in the subcontinent. Previous studies have highlighted the growing concern surrounding begomovirus-related infections in cucurbit crops, emphasizing the need for a comprehensive understanding of these viral pathogens in India (Nagendran *et al.*, 2017; Kumari *et al.*, 2021; Sahu *et al.*, 2021; Kumar *et al.*, 2021).

**Table 1. Primers used for the detection of Begomovirus infection in major cucurbits of Odisha**

Primer name	Type	Sequence	Targeting component	Amplification size (bp)	Annealing temp (°C)	Reference
Deng 540	Forward	TAATATTACCKG WKGVCSC	DNA A	~500	56	Deng <i>et al.</i> , 1994
Deng 541	Reverse	TGGACYTTRCAWGGBCCTTCACA				
PAL1c1960	Forward	ACNGGNAA RACNATGTGGGC	DNA A	~1200	55	Chatchawankanphanichand Maxwell, 2002
PAL1r772	Reverse	GGNAARTHGGATGGA				
GK ToLCV F	Forward	ATGKYGAAGCGACCAGCMGA	DNA A	~950	57	Nagendran <i>et al.</i> , 2014
GK ToLCV R	Reverse	CGCCCKCMGAYTGGGTTTTTCTT				
ToLCBD656F	Forward	CGCGTAAGGAAATCTGTGAAACAC	DNA B	~1200	55	Venkataravanappa <i>et al.</i> , 2012
ToLCBD971R	Reverse	GTTTCATGGTGGCGTTCTGCAAC				
OY2395F	Forward	GCTCCCTGAATGTTCCGGATGGA	DNA A	~1200	52	Venkataravanappa <i>et al.</i> , 2012
OY680R	Reverse	GTTTCRTCCATCCATATCTTAC				
Muni F4	Forward	ATATCTGCAGGGNAARATHGGATGGA	DNA A	~1200	58	Muniyappa <i>et al.</i> , 2000
Muni R5	Reverse	TGGACTGCAGACNGGNAARACNATGTGGGC				
BETA01	Forward	GGTACCACTACGCTACGCAGCAGCC	Betasatellite	~1300	57	Briddon <i>et al.</i> , 2002
BETA02	Reverse	GGTACCTACCCTCCAGGGGTACAC				



**Fig. 1. Symptom variations of begomovirus infection on different cucurbits in Odisha**

Begomoviruses, belonging to the family Geminiviridae, possess circular single-stranded DNAs with monopartite (DNA-A alone) or bipartite genomes (designated as DNA-A and DNA-B) transmitted by whitefly. The whitefly vector species in the *B. tabaci* complex vary in their host range, insecticide resistance, virus transmission, and capacity to cause plant disorders (Patil *et al.*, 2017; Czosnek *et al.*, 2017). Begomovirus diseases have been identified in various cucurbitaceous crops in India, including bitter gourd, ridge gourd, snake gourd, ash gourd, cucumber, ivy gourd bottle gourd, sponge gourd, and pumpkin, resulting in characteristic symptoms such as curling of leaf, mosaic mottling, crinkling, reduction in leaf size, yellowing, distortion, stunted growth, and blistering of leaves (Tiwari *et al.*, 2012; Hagen *et al.*, 2008; Nagendran *et al.*, 2016; Vignesh *et al.*, 2023). The impact of begomovirus infections on cucurbit is substantial, causing significant losses in India and world-wide (Sangeetha *et al.*, 2018; Sáez *et al.*, 2016).

In the survey conducted in Odisha from 2021 to 2023, a total of 320 samples from major cucurbits collected, revealing a diverse array of virus symptoms in the field. Common manifestations included mosaic patterns on leaves, yellow mosaic patches, leaf curling, blistering, leaf distortion, and leaf chlorosis (Fig. 1 and Table 2). Varied level of disease incidence was observed across various major horticultural crops like bitter gourd, bottle gourd, cucumber, pumpkin, ridge gourd, sponge gourd, snake gourd, and ash gourd. Percentage of disease

**TABLE 2: Symptoms of virus diseases of cucurbits in Odisha during 2021-2023**

SL. NO.	Place (District wise)	Location Coordinates	Crop	Symptoms observed
<b>Cuttack</b>				
1.	Banki	20.3766° N, 85.5290° E	Ridge gourd, Bitter gourd, Pumpkin	Mosaic leaf, crinkling of leaf, rough surface, leaf curling
2.	Nandailo	20.4515° N, 85.6705° E	Pumpkin, Cucumber	Yellow and dark green mosaic leaf, crinkling of leaf
3.	Ostia	20.4571° N, 85.6256° E	Ridge gourd	Mosaic of leaf, leaf curling, stunted growth of plant
4.	Jatamundia	20.4109° N, 85.6061° E	Bitter gourd	Leaf curling, reduction in plant height, upward folding
5.	Charigharia	20.4234° N, 85.6769° E	Bitter gourd, Pumpkin	Blistering of leaves, vein banding, leaf narrowing
6.	Kendupatna	20.4686° N, 86.1459° E	Ridge gourd, Cucumber	Light and dark green patches on the leaf surface, yellowing of leaves, vein clearing
7.	Tarito	20.4446° N, 86.0708° E	Ridge gourd, Cucumber	Leaf curling, crinkling of leaf, blistering of leaves
8.	Raghabpur	21.1776° N, 86.0568° E	Ridge gourd, Bitter gourd	Cupping of leaves, vein clearing, reduction in plant height, leaf narrowing
9.	Athagarh	20.5174° N, 85.6306° E	Ridge gourd, Pumpkin	Discolouration of leaves, upward folding of leaf, plant stunted growth
10.	Salipur	20.4843° N, 86.1192° E	Bitter gourd, Cucumber	Reduction in plant height and leaf size, inward curling,
<b>Puri</b>				
11.	Pipilli	20.1112° N, 85.8327° E	Bitter gourd, Ridge gourd	Leaf chlorosis, small leaflets, severe mosaic of leaf
12.	Siula	20.1530° N, 85.8678° E	Bottle gourd, Bitter gourd, Ridge gourd, Cucumber	Discolouration of leaves, upward folding of leaf, plant stunted growth
13.	Aruha	20.1401° N, 85.8531° E	Ridge gourd	Puckering of leaves, mosaic mottling of leaf, yellow mosaic patches
14.	Krushnapur	20.2119° N, 85.8422° E	Cucumber, Pumpkin	Leaf distortion, crinkling of leaf, blistering, vein banding
15.	Badalasan	20.0903° N, 85.9821° E	Pumpkin, Cucumber, Bitter gourd	Light and dark green patches on the leaf surface, severe mosaic of leaves
16.	Jagannathpur	20.3280° N, 85.9126° E	Bitter gourd, Cucumber	Upward and inward curling of leaves, cupping of leaves, chlorosis of leaf
<b>Jajpur</b>				
17.	Jagannathpur	20.8023° N, 86.1341° E	Pumpkin, Ridge gourd	Yellow mosaic patches, leaf distortion, cupping of leaves, chlorosis of leaf

18.	Sarbana	20.8086° N, 86.1298° E	Ridge gourd	Leaf distortion, vein clearing, reduction in plant height
19.	Harsinghapur	20.8209° N, 86.1332° E	Pumpkin	Yellow vein mosaic, blistering of leaves, leaf curling
20.	Andeiguda	20.8241° N, 86.1310° E	Bitter gourd, Pumpkin	Light and dark green patches on the leaf surface, leaf curling
21.	Karanjiari	20.8351° N, 86.1291° E	Ridge gourd	Leaf distortion, reduction in plant height, upward folding
22.	Mirzapur	20.8173° N, 86.2795° E	Pumpkin	Mosaic pattern on leaves, upward folding of leaves
23.	Kalaroi	21.0166° N, 85.7666° E	Bottle gourd, Ridge gourd, Cucumber, Pumpkin	Crinkling of leaf, discolouration of leaves, small leaflets
24.	Indaroi	20.7495° N, 86.2995° E	Pumpkin	Yellow mosaic patches, discolouration of leaves
25.	Kuakhai	20.3020° N, 85.8832° E	Pumpkin, Bitter gourd	Discolouration of leaves, plant stunted growth
<b>Kendrapada</b>				
26.	Bagada	20.4589° N, 86.4308° E	Bitter gourd, Ridge gourd, Pumpkin	Crinkling of leaf, yellow and dark green mosaic leaf
27.	Anandapur	20.4590° N, 86.4294° E	Ridge gourd	Light and dark green patches on the leaf surface, leaf curling
28.	Kalapada	20.4514° N, 86.4294° E	Ridge gourd, Cucumber, Bitter gourd, Pumpkin	Leaf distortion, plant stunted growth, inward curling
29.	Bahagandia	20.4467° N, 86.4227° E	Ridge gourd, Cucumber, Bitter gourd	Small leaf, yellow mosaic patches, leaf curling
30.	Karandia Patna	20.4471° N, 86.4191° E	Ridge gourd, Cucumber, Pumpkin	Crinkling of leaf, yellow and dark green mosaic leaf
31.	Naindipur	20.3895° N, 86.3882° E	Bottle gourd	Reduction in plant height and leaf size, inward curling
32.	Gardapur	20.3916° N, 86.3627° E	Cucumber, Pumpkin	Yellowing of leaves, vein clearing, plant stunted growth
<b>Khordha</b>				
33.	Balakati	20.2022° N, 85.8717° E	Bitter gourd, Ridge gourd	Cupping of leaves, vein clearing, reduction in plant height
34.	Kalyanpur	20.1704° N, 85.8717° E	Sponge gourd, Pumpkin	Mosaic pattern on leaves, upward folding of leaves
35.	Raghunathapur	20.3782° N, 85.8278° E	Bitter gourd, Pumpkin, Ridge gourd	Crinkling of leaf, mosaic of leaf, stunted growth of plant
36.	Aiginia	20.2509° N, 85.7811° E	Ash gourd, Bitter gourd, Ridge gourd	Mosaic of leaf, leaf curling, stunted growth of plant

<b>Naygarh</b>				
37.	Raipada	19.9759° N, 85.3730° E	Bitter gourd, Snake gourd	Reduction in plant height and leaf size, inward curling
38.	Balarampur	20.0645° N, 85.3524° E	Sponge gourd, Ridge gourd, Bitter gourd	Yellow mosaic patches, vein clearing, plant stunted growth
39.	Arjunapur	19.9590° N, 85.3511° E	Bottle gourd, Snake gourd, Bitter gourd	Yellow mosaic patches, leaf distortion, cupping of leaves, chlorosis of leaf
40.	Badakumari	20.1903° N, 85.3356° E	Pumpkin, Cucumber, Sponge gourd	Reduction in plant height and leaf size, inward curling
<b>Jagatsinghpur</b>				
41.	Kulasahi	20.3571°N, 86.1931°E	Cucumber, Bitter gourd, Pumpkin	Crinkling of leaf, cupping of leaves, chlorosis of leaf
42.	Odakhanda	20.3846°N, 86.2501°E	Ridge gourd, Bitter gourd, Pumpkin	Yellow mosaic patches, Leaf distortion, cupping of leaves, chlorosis of leaf
<b>Angul</b>				
43.	Chhendipada	21.0851°N, 84.8750°E	Pumpkin, Ash gourd, Ridge gourd	Reduction in plant height and leaf size, inward curling
44.	Bantala	20.7492°N, 85.3356°E	Cucumber, Pumpkin, Bottle gourd	Crinkling of leaf, upward and inward curling of leaves
45.	Mahidharpur	20.6942°N, 85.1803°E	Pumpkin, Ridge gourd, Sponge gourd	Yellow mosaic patches, leaf distortion, cupping of leaves, chlorosis of leaf
<b>Sundargarh</b>				
46.	Nuagaon	22.0221°N, 85.0979°E	Snake gourd, Ridge gourd, Bottle gourd	Leaf distortion, blistering of leaves, vein banding, leaf narrowing
47.	Biringatoli	22.2169°N, 84.4041°E	Pumpkin, Bottle gourd	Yellow mosaic patches, reduction in plant height and leaf size
48.	Jarda	21.7897°N, 84.5982°E	Sponge gourd, Bitter Gourd	Blistering of leaves, vein banding, leaf narrowing
49.	Anlaposhi	21.7769°N, 84.5720°E	Bitter gourd, Cucumber	Light and dark green patches on the leaf surface, crinkling of leaf
50.	Amasranga	22.0190°N, 84.1763°E	Bottle gourd, Pumpkin, Ridge gourd, Bitter gourd, Cucumber	Leaf distortion, blistering of leaves, vein banding,
51.	Saimal	21.9300°N, 83.9216°E	Sponge gourd, Ridge gourd, Pumpkin, Cucumber, Bitter gourd	Crinkling of leaf, blistering of leaves, vein banding, leaf narrowing,
<b>Sambalpur</b>				
52.	Ramjhali	21.7683°N, 84.4015°E	Cucumber, Snake gourd	Crinkling of leaf, blistering of leaves, vein banding, leaf narrowing

53.	Kuchinda	21.7428°N, 84.3489°E	Ridge gourd, Bitter gourd, Cucumber	Crinkling of leaf, yellow mosaic patches, vein clearing
54.	Petupali	21.2188°N, 83.2879°E	Pumpkin, Sponge gourd	Yellow mosaic patches, discolouration of leaves, small leaflets
55.	Talab	21.5533°N, 84.0055°E	Cucumber, Pumpkin, Bottle gourd	Reduction in plant height and leaf size, inward curling
<b>Bargarh</b>				
56.	Tentla	21.3923°N, 83.6056°E	Bitter gourd, Ridge gourd, Cucumber	Crinkling of leaf, cupping of leaves, vein clearing, reduction in plant height
57.	Dablong	21.4437°N, 83.5523°E	Bitter gourd, Cucumber,	Cupping of leaves, vein clearing, reduction in plant height
58.	Gopalpur	21.4535°N, 83.5763°E	Ridge gourd, Bitter gourd	Crinkling of leaf, yellow mosaic patches, vein clearing
59.	Khedapali	21.3966°N, 83.5870°E	Pumpkin, Ridge gourd, Bitter gourd	Crinkling of leaf, cupping of leaves, vein clearing, reduction in plant height
<b>Jharsuguda</b>				
60.	Pipilimal	21.7644°N, 83.7710°E	Pumpkin, Ridge gourd, Bitter gourd	Mosaic of leaf, leaf curling, stunted growth of plant
61.	Banjari	21.8199°N, 83.8305°E	Bitter gourd, Pumpkin, Ridge gourd	Yellow mosaic patches, discolouration of leaves, small leaflets
62.	H.Katapali	21.8913°N, 83.9564°E	Bitter Gourd, Ridge gourd	Crinkling of leaf, yellow mosaic patches, vein clearing
63.	Parmanpur	21.8035°N, 84.1738°E	Cucumber, Pumpkin	Light and dark green patches on the leaf surface, leaf distortion
64.	Remja	21.8383°N, 83.9365°E	Bitter gourd, Cucumber	Leaf distortion, leaf curling, stunted growth of plant
<b>Kalahandi</b>				
65.	Balrampur	19.9074°N, 83.1642°E	Ridge gourd, Bitter gourd	Reduction in plant height and leaf size, inward curling
66.	Kanakpur	19.9231°N, 83.1783°E	Bitter gourd, Cucumber	Yellow mosaic patches, light and dark green patches on the leaf surface
67.	Langigarh	19.7096°N, 83.3669°E	Pumpkin, Bitter gourd	Light and dark green patches on the leaf surface, leaf distortion
68.	Ichhapur	19.6410°N, 82.8896°E	Ridge gourd, Bitter gourd	Leaf distortion, leaf curling, stunted growth of plant
69.	Dumal	19.9750°N, 83.1408°E	Bitter gourd, Cucumber	Crinkling of leaf, discolouration of leaves, Leaf distortion
70.	Narla	20.0574°N, 83.3836°E	Bitter gourd, Bottle gourd, Pumpkin	Cupping of leaves, vein clearing, reduction in plant height

Nuapada				
71.	Kermeli	20.8150°N, 82.5456°E	Bitter gourd, Bottle gourd	Light and dark green patches on the leaf surface, leaf distortion
72.	Kodomeri	20.8167°N, 82.5332°E	Bitter gourd, Pumpkin, Cucumber	Leaf distortion, yellow and dark green mosaic leaf
73.	Darlimunda	19.9337°N, 83.1211°E	Ash gourd, Bitter gourd, Pumpkin, Ridge gourd	Crinkling of leaf, yellow mosaic patches, vein clearing

**Table 3. PCR-based detection of begomovirus in major cucurbits samples of Odisha**

District, Crop	Samples Collected	% of Disease Incidence	No of samples positive by PCR							% begomovirus infection
			Deng 540/541	PAL1c1960/PAL1r772	GKT0LCV F/R	ToLCBD656F/971R	OY2395F/OY680R	Muni F4/R5	Beta 01/02	
<b>Cuttack</b>										
Bitter gourd	6	85	5	4	6	5	4	2	0	100
Cucumber	5	79	5	4	4	4	3	3	1	100
Pumpkin	6	84	5	6	4	5	2	2	1	100
Ridge gourd	4	86	4	4	3	4	0	1	0	100
<b>Average Total</b>	<b>21</b>	<b>83</b>	<b>19</b>	<b>18</b>	<b>17</b>	<b>18</b>	<b>9</b>	<b>8</b>	<b>2</b>	<b>100</b>
<b>Puri</b>										
Pumpkin	5	91	5	3	4	5	2	2	1	100
Ridge gourd	5	49	4	4	3	4	1	2	2	80
Cucumber	3	88	2	3	3	1	0	0	0	100
Bitter gourd	6	93	5	6	5	5	4	4	0	100
Bottle gourd	3	55	2	3	1	3	1	0	0	100
<b>Average Total</b>	<b>22</b>	<b>75</b>	<b>18</b>	<b>19</b>	<b>16</b>	<b>18</b>	<b>8</b>	<b>8</b>	<b>3</b>	<b>96</b>
<b>Jajpur</b>										
Ridge gourd	5	73	4	5	4	5	3	2	1	100
Bitter gourd	4	47	4	4	4	4	3	3	0	100
Bottle gourd	3	70	3	2	3	2	1	1	0	100
Cucumber	4	73	3	4	4	4	0	0	0	100
Pumpkin	4	68	4	3	3	3	2	2	0	100
<b>Average Total</b>	<b>20</b>	<b>66</b>	<b>18</b>	<b>18</b>	<b>18</b>	<b>18</b>	<b>9</b>	<b>8</b>	<b>1</b>	<b>100</b>

Occurrence of begomovirus in cucurbits

Kendrapada										
Cucumber	4	93	4	3	4	4	3	2	1	100
Pumpkin	3	86	3	3	3	3	0	0	0	100
Bitter gourd	6	81	5	4	4	4	3	3	0	83.33
Bottle gourd	3	79	3	3	2	3	2	1	0	100
Ridge gourd	5	95	4	4	5	5	2	2	1	100
<b>Average Total</b>	<b>21</b>	<b>87</b>	<b>19</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>10</b>	<b>8</b>	<b>2</b>	<b>96.66</b>
Khordha										
Pumpkin	4	67	4	4	4	4	1	0	0	100
Ridge gourd	6	45	5	6	5	3	3	3	2	100
Sponge gourd	2	94	0	2	0	2	0	0	0	100
Bitter gourd	5	80	4	3	5	4	3	2	1	100
Ash gourd	3	76	2	2	2	2	0	0	0	66.66
<b>Average Total</b>	<b>20</b>	<b>72</b>	<b>15</b>	<b>17</b>	<b>16</b>	<b>15</b>	<b>7</b>	<b>5</b>	<b>3</b>	<b>93.33</b>
Naygarh										
Bitter gourd	5	74	4	5	4	4	2	1	0	100
Bottle gourd	3	83	3	3	3	2	0	1	0	100
Cucumber	5	73	5	4	3	5	0	0	0	100
Pumpkin	4	69	3	2	2	2	2	2	0	75
Ridge Gourd	6	82	5	4	5	4	3	3	0	83.33
Sponge gourd	2	92	2	2	2	2	0	0	0	100
Snake gourd	2	87	0	2	0	1	1	0	0	100
<b>Average Total</b>	<b>27</b>	<b>80</b>	<b>22</b>	<b>22</b>	<b>19</b>	<b>20</b>	<b>8</b>	<b>7</b>	<b>0</b>	<b>94.04</b>
Jagatsinghpur										
Cucumber	6	71	5	4	0	4	3	0	0	83.33
Bitter gourd	4	68	3	3	2	3	2	2	0	75
Pumpkin	5	84	4	5	3	4	2	1	0	100
Ridge gourd	5	90	4	4	0	3	3	2	0	80
<b>Average Total</b>	<b>20</b>	<b>78</b>	<b>16</b>	<b>16</b>	<b>5</b>	<b>14</b>	<b>10</b>	<b>5</b>	<b>0</b>	<b>84.58</b>

Angul										
Pumpkin	4	72	3	4	3	4	2	1	1	100
Ash gourd	3	58	3	3	2	3	1	1	0	100
Ridge Gourd	5	90	4	5	4	5	2	1	2	100
Cucumber	4	81	3	3	4	3	3	2	2	100
Bottle gourd	3	68	0	2	2	2	1	0	0	66.66
Sponge gourd	3	77	3	0	2	3	2	1	0	100
<b>Average Total</b>	<b>22</b>	<b>74</b>	<b>16</b>	<b>17</b>	<b>17</b>	<b>20</b>	<b>11</b>	<b>6</b>	<b>5</b>	<b>94.44</b>
Sundargarh										
Bottle gourd	3	71	2	3	2	3	2	2	1	100
Cucumber	5	89	4	5	4	5	0	0	0	100
Pumpkin	4	67	3	4	3	4	2	3	2	100
Ridge gourd	5	93	4	3	5	4	2	0	2	100
Sponge gourd	2	40	0	2	0	2	2	0	0	100
Snake gourd	3	62	2	2	1	3	1	1	1	100
Bitter gourd	6	87	5	6	4	4	0	0	0	100
<b>Average Total</b>	<b>28</b>	<b>73</b>	<b>20</b>	<b>25</b>	<b>19</b>	<b>25</b>	<b>9</b>	<b>6</b>	<b>6</b>	<b>100</b>
Sambalpur										
Pumpkin	5	54	3	5	4	4	3	0	2	100
Ridge gourd	6	88	4	5	4	4	2	2	1	83.33
Cucumber	4	56	4	3	4	3	0	2	0	100
Bitter gourd	7	94	5	6	5	6	3	0	1	85.71
Bottle gourd	3	56	2	3	2	3	0	0	0	100
Sponge gourd	2	86	1	2	2	2	1	0	0	100
Snake gourd	3	35	3	3	1	2	2	1	1	100
<b>Average Total</b>	<b>30</b>	<b>67</b>	<b>22</b>	<b>27</b>	<b>22</b>	<b>24</b>	<b>11</b>	<b>5</b>	<b>5</b>	<b>95.57</b>

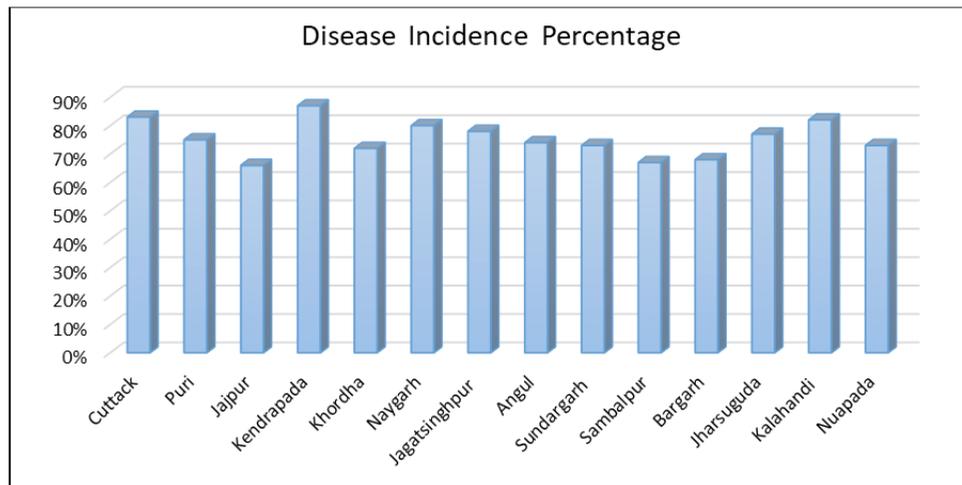
Occurrence of begomovirus in cucurbits

Bargarh										
Cucumber	6	83	5	6	6	4	2	2	1	100
Bitter gourd	5	41	4	5	4	5	0	0	0	100
Pumpkin	5	64	3	4	3	4	2	3	2	80
Ridge gourd	4	86	4	3	4	4	2	0	2	100
<b>Average Total</b>	<b>20</b>	<b>68</b>	<b>16</b>	<b>18</b>	<b>17</b>	<b>17</b>	<b>6</b>	<b>5</b>	<b>5</b>	<b>95</b>
Jharsuguda										
Pumpkin	5	92	4	5	4	4	2	2	1	100
Ridge gourd	4	65	4	3	4	3	0	2	0	100
Cucumber	5	89	5	4	5	4	3	0	1	100
Bitter gourd	6	64	5	6	6	5	2	0	0	100
<b>Average Total</b>	<b>20</b>	<b>77</b>	<b>18</b>	<b>18</b>	<b>19</b>	<b>16</b>	<b>7</b>	<b>4</b>	<b>2</b>	<b>100</b>
Kalahandi										
Ridge gourd	5	87	3	5	4	4	3	0	2	100
Bitter gourd	6	79	4	5	4	4	2	2	1	83.33
Bottle gourd	3	59	3	3	3	3	0	0	0	100
Cucumber	4	95	2	4	2	4	1	0	1	100
Pumpkin	5	88	3	4	4	4	1	1	0	80
<b>Average Total</b>	<b>23</b>	<b>82</b>	<b>15</b>	<b>21</b>	<b>17</b>	<b>19</b>	<b>7</b>	<b>3</b>	<b>4</b>	<b>92.66</b>
Nuapada										
Bottle gourd	3	74	2	2	2	2	1	1	0	66.66
Cucumber	4	54	3	4	3	3	0	0	0	100
Pumpkin	5	66	5	4	4	5	2	2	1	100
Bitter gourd	5	87	4	4	3	4	2	1	0	80
Ridge gourd	6	90	5	4	6	5	2	2	0	100
Ash gourd	3	68	2	3	2	3	0	0	0	100
<b>Average Total</b>	<b>26</b>	<b>73</b>	<b>21</b>	<b>21</b>	<b>20</b>	<b>22</b>	<b>7</b>	<b>6</b>	<b>1</b>	<b>91.11</b>

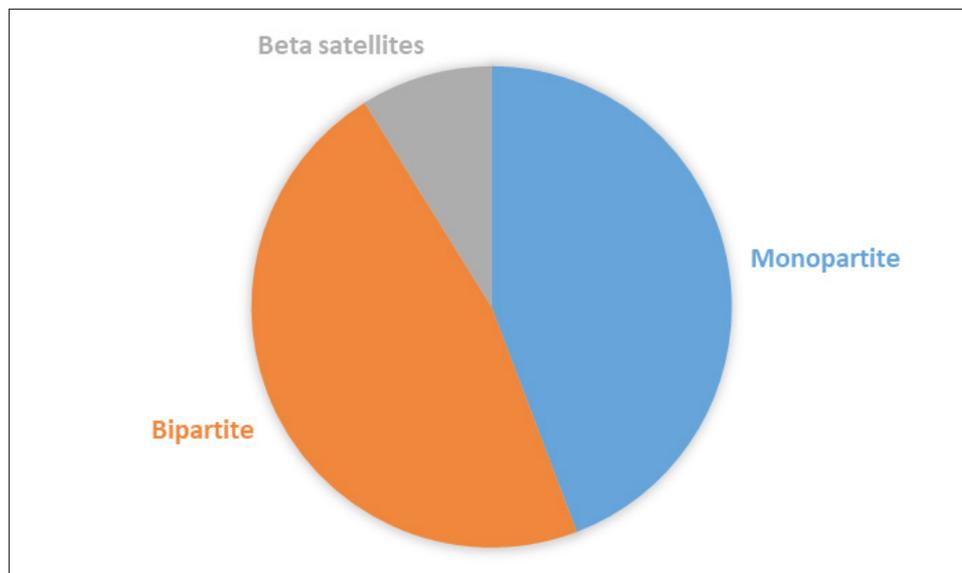
incidence were varied in different locations, *viz.*, in Cuttack (83%), Puri (75%), Jajpur (66%), Kendrapada (87%), Khordha (72%), Naygarh (80%), Jagatsinghpur (78%), Angul (74%), Sundargarh (73%), Sambalpur (67%), Bargarh (68%), Jharsuguda (77%), Kalahandi (82%), and Nuapada (73%) respectively (Table 3; Fig 2).

DNA extractions were performed for 320 viral DNA samples, and DNA concentration was measured using a Nano spectrophotometer. The concentration of DNA varied between 30 and 1200 ng/ $\mu$ l depending on the sample. In a similar study, quantification of viral DNA in chilli revealed concentrations ranging from 188.49 to 475.07 ng/ $\mu$ l (Nigam *et al.*, 2015). For begomovirus detection, the PCR method utilizing universal degenerate primers and genus-specific primers for different genomic

components were employed. The results revealed the infection of begomovirus in various cucurbit crops, with high incidence rates across the districts of Odisha. A total of 320 samples underwent PCR diagnosis. Among the samples tested, 265 were successfully amplified using the ToLCBD656F/ToLCBD971R primer pair, which targeted the homologous region in the DNA B segment. Beta-satellites are the helper DNA component play a crucial role in exacerbating symptoms in infected plants, leading to severe manifestations such as enation, leaf malformation twisting, and stunting (Singh *et al.*, 2021). Out of all the collected cucurbits samples, 68 bitter gourd (75.38%), 26 bottle gourd (68.33%), 59 cucumber (78.76%), 67 pumpkin (75.14%), 66 ridge gourd (79.92%), 11 sponge gourd (77.80%), 8 snake gourd (61.30%), and 9 ash gourd (67.30%) were



**Fig. 2.** Average percent disease incidence of Begomovirus in different district of Odisha state on cucurbits.



**Fig. 3.** Percentage of samples infected with Begomovirus in cucurbitaceous crops determined by PCR assay.

found to be infected with Begomovirus. Specifically, 39 out of 320 were detected with the presence of betasatellites. This finding underscores the significance of betasatellites in the pathogenesis of begomoviruses and highlights their potential impact on cucurbits. The present study corroborates findings from previous research, highlighting the dominance of begomoviruses in cucurbit samples collected from southern India (Nagendran *et al.*, 2017). Additionally, similar studies reported high incidences of begomovirus infections in cucurbits across different regions (Kumari *et al.*, 2021). Several bipartite begomoviruses such as ToLCNDV, SLCCNV, ToLCPaIV, squash leaf curl Philippines virus, etc were documented from different parts of the globe on cucurbits (Kumari *et al.*, 2022). Likewise in the current study, among all collected samples, 255 samples were amplified with Deng 540/Deng 541 primers accounting to 79.68 percent, likewise 274 samples with PALIc1960/PALIr772 (85.62%), 240 samples with GK ToLCV F/GKToLCVR (75%), 265 samples with ToLCBD656F/ToLCBD971R (82.81%), 119 samples with OY2395F/OY680R (37.18%), 84 samples with Muni F4/Muni R5 (26.25%), and 39 samples with BETA01/BETA02 primer pairs (12.18%). PCR-based results among tested samples revealed varying degrees of begomovirus infection in different districts, as detailed in Table 3 *viz.*, Cuttack (100%), Puri (96%), Jajpur (100%), Kendrapada (96.66%), Khordha (93.33%), Nayagarh (94.04%), Jagatsinghpur (84.58%), Angul (94.44%), Sundargarh (100%), Sambalpur (95.57%), Bargarh (95%), Jharsuguda (100%), Kalahandi (92.66%), and Nuapada (91.11%) respectively (Table 3). The study also aligns with the broader global perspective on begomovirus-induced diseases in cucurbits (Leke *et al.*, 2015; Camara *et al.*, 2013). The variation in disease incidence across districts and regions can be attributed to differences in weather conditions, cropping patterns, vector pressure at the location, and management practices adopted by farmers.

## CONCLUSION

In summary, the rising threat of begomovirus infections poses a significant challenge to India's agriculture. The study's exploration of begomovirus genomics, the vector whitefly, and associated symptoms offers crucial insights for effective disease management. The research underscores the widespread prevalence of begomovirus infection in cucurbit crops in the state of Odisha, stressing the importance of targeted control

measures. The substantial impact on crop yields highlights the necessity for continuous surveillance and proactive measures to protect cucurbit production. A collaborative effort among researchers, farmers, and policymakers is imperative for developing sustainable solutions and minimizing economic losses from begomovirus infection in cucurbits.

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## AUTHOR CONTRIBUTION

GS, AGC and PS conceptualized the study. DP and MP conducted the field surveys, sample collection, and laboratory-based experiments. AR and NK performed the molecular analyses and data interpretation. The first draft of the manuscript was prepared by DP and GS. All authors have read and agreed to the published version of the manuscript.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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