



OCCURRENCE AND DISTRIBUTION OF VIRAL DISEASES OF BITTERGOURD (*MOMORDICA CHARANTIA* L.) IN MAJOR CULTIVATED AREAS OF KERALA

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Abstract

In the survey conducted at five locations in Thiruvananthapuram district, Pappanchani area recorded highest incidence of viral disease (60%) while highest Vulnerability Index (V.I) was recorded from Vellayani (56.00). In Idukki district, six major bittergourd cultivating areas were surveyed among which Rajakumary area recorded the highest disease incidence (100%) and V.I (82.00). In Palakkad district, five locations were surveyed, among which Panackatri and Thekkepotta recorded highest disease incidence of 88% and highest V.I (69.00). Symptoms associated with the disease include yellow mottling, mosaic, blistering, leaf curl and reduction in leaf size. Yellow mosaic and blistering is also seen in case of severe infection finally leading to stunting of the plant, reduced flowering and fruiting and hairyness on the stem. Enzyme Linked Immunosorbent Assay (ELISA) and Dot Immunobinding Assay (DIBA) revealed the presence of three viruses belonging to Begomo, CMV and POTY group causing a mixed infection in bittergourd.

Keywords: Survey, Symptomatology, bittergourd viral disease, ELISA, DIBA

I. INTRODUCTION

Bitter melon, bittergourd, bitter squash, or balsam-pear (*Momordica charantia* L.), is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. In Kerala, it is a popular cucurbitaceous vegetable cultivated in an area of 2673 ha and maximum area under cultivation is in Idukki district (499 ha) followed by Palakkad district (372 ha) [1].

Virus diseases are a worldwide problem and a major limiting factor for production in cucurbits. Geminiviruses transmitted by the whitefly *Bemisia tabaci* (Gennadius) are wide spread in tropical and subtropical regions of the world. More than 50 geminiviruses have been reported to be transmitted by whiteflies [2,3]. The association of Bittergourd distortion mosaic virus (BDMV) with bittergourd was first reported from Kerala [4]. An aphid-transmitted bittergourd mosaic virus was reported by [5] and a *Begomovirus* infection in bitter melon has been reported from Lucknow, India by [6]. Symptoms of mosaic disease in bittergourd in Kerala were also reported [8]. Association of *Indian Cassava Mosaic virus* (ICMV) with yellow mosaic of bittergourd was reported [7].

Not much work has been undertaken to study the distribution of the viruses in bittergourd in Kerala and hence a survey was carried out during the major seasons of cultivation in three districts of Kerala with the maximum area under cultivation to study the occurrence and distribution of the viral disease.

II. MATERIALS AND METHODS

A) Survey

Survey was conducted in three districts of Kerala viz., Thiruvananthapuram, Idukki and Palakkad selected based on the extent area of under cultivation and geographical zones. The survey was conducted in the major bittergourd growing seasons in each district during 2014-2016. The list of cultivated area was prepared in consultation with Vegetable and Fruit Promotion Keralam field officials. Simple random sampling was adopted and 40 sampling units (farmers) per district was selected. Stage of the crop for observation was the twinning stage before it in the pandal. Minimum plot size for sampling was 50 cents and 25 plants was selected at random and scored. Disease incidence and Vulnerability Index (V.I) were calculated. Expressions of symptoms were scored according to the 0-5 scale developed by [9] with slight modifications made for scoring the viral disease in bittergourd.

- 0 - no symptom
- 1 - Very light mottling
- 2 - mottling with dark green and yellow colour
- 3 - blisters and raised surface on leaves
- 4 - distortion of leaves, curling, hairyness, reduction in leaf size
- 5 - stunting with negligible or no flowering and fruiting or very small fruits

Based on the rating Vulnerability Index was calculated using the formula,

$$V.I = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5) \times 100}{n_t (n_c - 1)}$$

- V.I - Vulnerability index
- n_0, n_1, \dots, n_5 - number of plants in the category of 0, 1, 2, 3, 4, 5
- n_t - total number of plants
- n_c - total number of categories

Percent disease incidence

Percent disease incidence was calculated as

$$\text{Disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

B) Symptomatology

The symptoms associated with the viral disease were collected from farmers' field and studied. *In vitro* maintenance of viruses was mainly done by wedge grafting the infected scion onto 3-5 leaf staged bittergourd seedlings and the graft union clipped with a graft clip. The symptoms of transmission were obtained after 10 days of inoculation.

C) DAC-ELISA

1. ELISA was carried out to detect the presence of viruses in infected bittergourd leaves. The procedure described by [10] was followed for the detection. ELISA was carried out to detect the presence of viruses in infected bittergourd leaves. One gram infected leaf was homogenized in 5 ml of coating buffer (carbonate buffer) containing 2 per cent (w/v) PVP under chilled condition. Healthy plant extract was prepared by using leaves of healthy plants. The homogenate was centrifuged at 5000rpm for 15 min at 4°C.
2. The supernatant from the centrifuge was dispensed in to immunological plates at the rate of 100 µl. The treatments were replicated twice and incubated for 1 h at 37°C.
3. The wells were washed with Phosphate buffer Saline-Tween (PBS-T) three times each for duration of 3 min using an ELISA plate washer (pw-40, BIORAD).

4. Blocking was done with 100 µl of three per cent spray dried milk powder (SDM) for 1h at 37⁰C. After incubation plates were washed with PBS-T as before.
5. Then plates were treated with 100 µl of polyclonal antibodies, diluted (1:200) in PBS-T with Polyvinyl pyrrolidone (2%) and egg ovalbumin (0.2%) to form PBS-TPO. Two replications were maintained for each treatment and incubated overnight at 4⁰C/ or kept at 37⁰C for 2h. The plates were washed again with PBS-T.
6. This was followed by treatment with 100 µl of 1:10000 diluted anti rabbit immunoglobulin (SIGMA-Aldrich) in PBS-TPO and incubated for 2 h at 37⁰C. Wells were washed with PBS-T as before.
7. The substrate p-nitro phenyl phosphate in diethanolamine buffer (1mg per ml) was added to each well (100 µl per well) and incubated for 1 h at 37⁰C. Reaction was stopped by adding 50 µl of four per cent sodium hydroxide.

The absorbance was read at 405nm in an ELISA reader (Microplate Reader 680, BIORAD).

III. RESULT AND DISCUSSION

In the survey conducted at five locations in Thiruvananthapuram district , Pappanchani area recorded highest incidence of viral disease (60%) while highest Vulnerability Index (V.I) was recorded from Vellayani (56.00). In Idukki district, six major bittergourd cultivating areas were surveyed among which Rajakumary area recorded the highest disease incidence (100%) and V.I (82.00). In Palakkad district, five locations were surveyed, among which Panackatri and Thekkepotta recorded highest disease incidence of 88% and highest V.I (69.00)(Table1,2,3) .Symptoms associated with the disease include yellow mottle, mosaic, blistering , leaf curl and reduction in leaf size ,yellow mosaic and blistering is also seen in severe infection finally leading to stunting of the plant, reduced flowering and fruiting ,and hairyness on stem. Enzyme Linked Immunosorbent Assay (ELISA) revealed the presence of three viruses belonging to Begomo, CMV and POTY group causing a mixed infection in bittergourd in all the three locations as the absorbance values of samples in all the districts show an increase of 1.5 times more than the healthy except the reaction of sample from Idukki district to POTY antiserum which indicates the presence of Begomo and CMV infection only. The major variety that was grown in Thiruvananthapuram and Idukki was Var. Preethi while the Palakkad farmers from Panackatri were following open precision system and the hybrid Maya was popular among them.

In India, a whitefly transmitted *Bittergourd distortion mosaic virus* (BDMV) producing mosaic, reduced leaf size, curling, deformed fruits, thick and hairy stem was reported [11]. [12] reported major economic loss due to cucurbit viral diseases in Lebanon. A survey conducted revealed the presence of *Zucchini yellow mosaic Potyvirus* (ZYMV) and *Cucurbit aphid-borne yellows Polerovirus* (CABYV) are the most common viruses followed by *Watermelon mosaic Potyvirus* (WMV), *Papaya ringspot Potyvirus*-watermelon strain (PRSV-W) and to a lesser extent *Cucumber mosaic Cucumovirus* (CMV). The occurrence of yellow mosaic disease of bitter gourd in Tamil Nadu caused by *Indian cassava mosaic virus* (ICMV) has been reported [7] as mosaic and mottling on young leaves. The mottling usually started at the edges of the leaf and advanced inwards subsequently, chlorotic patches appeared on leaves and in advanced stages of infection, the entire leaf becomes chlorotic with few, small patches of green tissue remaining over the leaf area. The occurrence of seven viruses in cucumber from major cultivated area of Okalhoma was reported [13] . DAC – ELISA has been used for the confirmation of virus mixtures in bittergourd and indicator plants [14].

IV. CONCLUSION

Thus the present study indicates the presence of three viruses in varying proportions causing yellowing, mosaic, curling and stunting of plants affecting the yield and great loss to farmers and is found in all the areas of cultivation as Begomo is whitefly borne while CMV and POTY is aphid borne.

Continuous cultivation, presence of insect vectors and suitable environmental conditions for the multiplication of insect vectors make these viruses a persistent field problem.

Table 1. Distribution , incidence and Vulnerability Index of bittergourd plants to viruses in Tiruvananthapuram district

Sl.No.	Location	GPS coordinates	Season	Disease Incidence (%)	Vulnerability Index	Insects associated
I	District:Thiruvananthapuram					
1.	Pappanchani	N 8°26'20.21" E 76°58'32.98"	June-September2015	4-60	8-36	Mites -20% Aphids 10%
2.	Palapoor	N 08°25'43.2" E 76°58'57.1"	June-September2015	22	28	Jassids-10% Aphids-10%
3.	Kalliyoor	N 08°25'43.2" E76°58'57.1"	June-September2015	32-42	29-48	Aphids-20% Whiteflies-10%
4.	Karakulam	N 8°34'1.96" E 76°59'24.82"	June-September2015	10-28	8-22	Aphids-10%
5.	Vellayani College	N 08°25'45.97" E 76°59'16.84"	Year round	55	56	Aphids-20% Mites-20% Jassids-20% Whiteflies-24%

Table 2. Distribution , incidence and Vulnerability Index of bittergourd plants to viruses in Palakkad district

Sl.No.	Location	GPS coordinates	Season	Disease incidence (%)	Vulnerability Index
II	District: Palakkad				
1.	Puthucode thekkepotta	N 10°38'18.1" E76°26'13.82"	April-September2015	4-88	4-69
2.	Panackatti	N 10°35'16.4" E 76°39'13.8"	July-October 2015	60-88	14-57
3.	Attayanpathy	N 10°46'10.0" E 76°51'43.9"	June-December2015	60-80	19-28
4.	Kudalloor	N 10°45'43.3" E 76°51'27.0"	June-December 2015	80	25-31
5.	Vithanaserry	N 10°35'59.95" E 76°37'0.20"	June-December 2015	80	29

Table 3. Distribution, Incidence and Vulnerability Index of bittergourd plants to viruses in Idukki district

Sl.No	Location	GPS coordinates	Season	Disease incidence (%)	Vulnerability Index
II	District: Idukki				
1.	Rajakumary	N 9°57'52.9" E 77°08'00.7"	September- January 2015	10-100	10-82
2.	Rajakkad	N 9°58'04.4" E 77°03'46.8"	September- January 2015	24-90	16-76
3.	Konnathady	N 9°56'17.7" E 77°01'47.7"	September- January 2016	52-82	15-31
4.	Parathodu	N 9°56'24.18" E 77°01'52.53"	September- January 2016	48-80	31-36
5.	Thoprankudy	N 9°52'33.8" E 77°03'21.3"	September- January 2016	24-80	11-32
6.	Chembakapara	N 9°54'07.2" E 77°03'21.5"	September- January 2016	80	27

Table 4. Detection of Begomo, CMV and POTY viruses in infected leaf samples of bittergourd in different locations

District	Begomo (A ₄₀₅ value)	Increase In absorbance	CMV (A ₄₀₅ value)	Increase In absorbance	POTY (A ₄₀₅ value)	Increase In absorbance
Thiruvananthapuram	0.402 (0.180)	2.23 times	0.077 (0.044)	19.25 times	0.044 (0.025)	1.76 times
Idukki	1.335 (0.426)	3.13 times	0.310 (0.032)	9.69 times	0.081 (0.064)	1.27 times
Palakkad	0.390 (0.180)	2.16 times	0.064 (0.007)	9.14 times	0.058 (0.039)	1.49 times



Plate 1. Leafcurl and blistering

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