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Serological Detection of Some Viruses Causing Virus like Symptoms in Major Cucurbit Crops Grown in Sri Lanka

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Abstract: Most cucurbits crop grown in Sri Lanka are adversely suffered from virus like symptoms causing substantial yield losses. This condition leads to decline the economic gains of cucurbit growers and impede expansion and intensification of cultivation. Number of leaf sample of different cucurbit crops i.e. bitter gourd (*Momordica charantia*), snake gourd (*Trichosanthes cucumerina*), water melone (*Citrullus lanatus*), pumpkin (*Cucurbita maxima*), luffa (*Luffa acutangula*) and cucumbers (*Cucumis sativus*) showing virus like symptoms collected from major cucurbit growing area of Sri Lanka were assayed for the presence of viruses by DAS-ELISA using commercial polyclonal antibodies developed by agdia Inc. Ltd., USA for Cucumber Mosaic Virus (CMV), Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ring Spot Virus (PRSV), Cucumber Green Mottled Mosaic Virus (CGMMV), Tobacco Ring Spot Virus (TRSV) and Tomato Ring Spot Virus (ToRSV). Highly diversed symptoms including leaf mottling, leaf mosaic, leaf distortion, leaf curling, reduced leaf size, rosetting and stunting were observed in virus infected samples. Among the viruses detected CMV is the most prevalent virus affecting cucurbits in Sri Lanka. Furthermore CGMMV, TRSV, ToRSV, PRSV and ZYMV were found in infected cucurbits. Seed transmitted CGMMV was found from bitter gourd, pumpkin and cucumber. Also, PRSV was found to be the most common virus in bitter gourd and showing symptoms such as yellowing of leaves, small circular patches on leaves, yellowing of pods and oily spots on pod. CMV was prevalent in luffa, cucumber and snake gourd. Out of 238 cucurbit samples, only 74 samples were positive serologically presence of viruses indicating that, there are undetectable viruses other than above six viruses.

Keywords: Cucurbits, ELISA, Symptomatology, Viruses.

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1. Introduction

Cucurbits are popular vegetables belonging to the family cucurbitaceae and cultivated in different agro-ecological zones in Sri Lanka. Most cucurbit varieties grown in Sri Lanka suffer from virus like symptoms, which include leaf mottling, mosaic, yellowing distortion of leaves, rosette and stunting of plants. Such kind of symptoms caused significant yield losses in bitter gourds, snake gourds, luffa, water melons, pumpkins and cucumbers (personal communication with Agriculture extention officers).

Virus diseases are the most common cause affecting cucurbits (Alonso *et al.*, 1997). Approximately more than 35 viruses have been isolated from the family cucurbitaceae (Mehmet *et al.*, 2006). Serkan and Filiz (2013) reported the virus found in cucurbits in Turkey as Cucumber Mosaic Virus (CMV), Squash Mosaic Virus (SqMV), Water Melon Virus (WMV) and Zucchini Yellow Mosaic Virus (ZYMV). It has reported that the most prevalent viruses are CMV, PRSV, WMV and ZYMV and the most common virus in cucurbits is CMV (https://wike.bugwood.org). Ariyarathne *et al.*, (2005) reported that most important viruses identified in snake gourd is CMV in Sri Lanka.

Many symptoms are not virus specific and may be caused by other agents such as insects, fungal or bacterial pathogens or be the result of deficiency, genetic abnormalities (leaf variegation) or aging of the plant (Agrios, 1988). Cucurbits infected with sucking insects and mites may show virus like symptoms which obtain lead to misdiagnosis as viral infection (personal communication). Some plants infected with viruses can appear healthy and symptoms expression may depend on the environmental conditions. Thus, symptomatology is essential for description of a virus disease. It is usually not suitable for diagnosis or identification of virus diseases (Agrios, 1988).

Viruses transmit through different mode of transmissions. Most of viruses are spread or vectored by insects. CMV, PRSV, WMV, ZYMV, TRSV (Tobacco Ring Spot Virus) are transmitted by aphids (Provividenti, 1996). Further, TRSV can also be transmitted by mites, thrips, and nematodes. CMV can easily move from one plant to another by just touching of a hand or carryover of sap on farming tool. CGMMV, TRSV, SqMV can transmit in cucurbits through seeds (Provividenti 1996; Romay *et al.*, 2014; Sue Tolin, 2014).

There are numerous ways to control viral diseases. However correct diagnosis of viral disease is a perquisite for the development of effective and sustainable management strategies (Agrios, 1988; Mehmet *et al.*, 2006). Serological assays known as enzyme-linked immunosorbent assay (ELISA) can be used to diagnosis of viral infected plant samples accurately. It is relatively rapid and sensible and requires only a small amount of anti-body (Agrios, 1988, https://www.mybiosource.com2006).With the background of this information, along term experiment was planned with the objective of studying the symptomatology of cucurbit viral disease and to detect the possible viruses associated with the virus like symptoms in major cucurbits grown in Sri Lanka through ELISA.

2. Materials and Methods

2.1 Collection of virus infected cucurbit samples

Samples of different cucurbit crops showing virus like symptoms were collected from major cucurbit growing areas of Sri Lanka. Cucurbit samples suspected to be infected with virus were dispatched of plant pathology laboratory at Horticulture Crop Research and Development Institute, Gannoruwa, Sri Lanka for detection of CMV, PRSV, CGMMV, ToRSV, TRSV and ZYMV.

2.2 Symptomology

Major external symptoms of each sample was recorded either at the time of collection or at the time of receipt using the terminology given by Dijkestra and de Jager (1998).

2.3 Serological detection of viruses through ELISA

Each sample immediately after collection were subjected to serological assay through standard (direct) double antibody sandwitch (DAS) enzyme-linked immunosorbant assay (ELISA) to detect the possible infection of sample with viruses. All laboratory experiment for DAS-ELISA were carried out at Division of Plant Pathology, Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka. DAS-ELISA for different cucurbit crop samples were performed using commercial antibodies (Agdia Inc. Ltd, USA) for CMV, PRSV, ZYMV, CGMMV, TRSV and ToRSV according to the protocols given by Agdia Inc. Ltd, USA.

3. Results and Discussion

Total number of 238 symptomatic cucurbit samples which had been suspected to be infected with viruses was collected from major cucurbit growing areas of Sri Lanka including central and north central province. The spectrum of samples consisted of cucurbit crops such as bitter gourd (*Momordica charantia*), snake gourd (*Trichosanthes cucumerina*), pumpkin (*Cucurbita maxima*), luffa (*Luffa acutangula*), cucumbers (*Cucumis sativus*) and water melon (*Colocynthis citrullus*). All cucurbit samples were tested for the presence of viruses by DAS-ELISA using commercial polyclonal antibodies purchased from Agdia Inc Ltd, USA. Total number of 238 cucurbit crop samples showing virus like symptoms were tested for possible virus infection by DAS-ELISA summarized results are shown in Table 1, out of 238 samples, 74 samples showed positive reaction for ELISA.

Table 1. Detection of different viruses in cucurbit crops through DAS-ELISA

Virus	Number of samples react positively
CMV	41
PRSV	21
ZYMV	01
CGMMV	05
TRSV	04
ToRSV	02
Total	74

Note: All leaf samples were symptomatic and suspected to be infected with viruses.

Out of cucurbit samples assayed for CMV, 41 samples showed positive reactions becoming the CMV the most prevalent virus in pumpkin, luffa and cucumbers and PRSV in bitter gourd (Table 1 & 2). Ariyarathne *et al.*, (2005) stated that the CMV was the most prevalent virus affecting snake gourd in Sri Lanka. Out of 74 positively react samples, CGMMV, TRSV, ToRSV and ZYMV were detected in 05, 04, 02 and 01 samples respectively. It has been reported that many virus association of cucurbit cultivation in many countries. Cucurbits are known to infect normally more than 35 viruses (Mehmet *et al.*, 2006; Romay *et al.*, 2014) suggesting the possibility of infecting samples with other viruses which were not considered in this experiment. Table 2 shows the prevalent of different viruses among cucurbit crops and their major symptoms. Highest number of samples of 58 was tested in bitter gourd for all 6 viruses followed by 56 in pumpkins, 51 in water melon, 32 in luffa, 31 in cucumbers and 10 in snake gourd.

Table 2. Prevalent of different viruses among cucurbit crops and respective major symptoms

Cucurbit crops	Total number of samples assayed	ELISA test	No: of positive samples	Major symptoms observed on samples react positively
Bitter gourd	58	CMV	5	Mottling/mosaic/leaf distortion/downward curling/rosetting
		PRSV	16	Mosaic/leaf distortion/vein clesring/stunting/rosetting/ marginal yellowing/oily spots on pods
		CGMM	2	Mosaic/shoe string/rosetting
		TRSV	2	Mosaic/leaf distortion/upward curling/reduced leaf size/rosetting
		ToRSV	2	Mild mosaic
		CMV TRSV CGMMV	1	Mosaic/rosetting/green colour patches on leaves
		CMV TRSV	1	Mottling/mosaic/leaf distortion
pumpkin	56	CMV	9	Mottling/leaf distortion/stunting
		PRSV	2	
		ZYMV	1	Severe mosaic/leaf distortion/reduced leaf size
		CGMMV	1	Mosaic/rosetting/green colour patches on leaves
Luffa	32	CMV	9	Mottling/mosaic/leaf distortion/downward curling/stunting/thickening of leaves
		CMV TRSV CGMMV	1	Mottling/mosaic/leaf distortion
Water melon	51	CMV	1	Mosaic/reduced leaf size/stunting
		CGMMV	1	Mosaic/shoe string/rosetting
Snake gourd	10	CMV	4	Mosaic/reduced leaf size/downward curling/rostting/thickening of leaves
		PRSV	1	Yellowing of leaves/oily spots on pods
Cucumber	31	CMV	09	Mottling/leaf distortion/stunting//rostting
		CMV PRSV	2	
		CGMMV	1	Mosaic/leaf

		PRSV		distortion/stunting/dark green coloration
		CMV PRSV TRSV	1	Severe mosaic/stunting
		CGMMV PRSV	1	Severe mosaic/stunting
		CGMMV PRSV TRSV	1	Mosaic/leaf distortion/stunting/dark green coloration
Total	238			

CMV was the most prevalent virus found in cucumber, luffa and pumpkin accounting higher percent of ELISA positive samples. Ariyarathne *et al.,* (2005) stated the CMV was the most common virus on snake gourd in Sri Lanka. However PRSV was the most frequently found virus particularly in bitter gourd according to the data gathered from present study.

Among the 74 ELISA positive samples, only one sample of pumpkin gave positive reaction for ZYMV while others were negative. It suggests that ZYMV is not much important virus in cucurbits at present in Sri Lanka. However ZYMV is one of the most aggressive and destructive to virus of cucurbits in some countries (Serkan and Filiz, 2013). Leaf samples of symptomatic cucumbers were positive for CMV, PRSV, CGMMV and TRSV. CMV was detected in 11 positive samples while PRSV in 3 positive samples and CGMMV in 1 positive sample and TRSV in 1 positive sample. Varity of symptoms was observed in cucurbit samples which gave positive reactions for tested viruses. Major symptoms include yellowing, mottling, mosaic, downward curling of leaves, upward curling of leaves, leaf distortion, reduced leaf size, rosetting and stunting of plants (Table 2). Serkan and Filiz (2013) found that pumpkin, squash, zucchini and cucumber were infected with ZYMV showing severe stunting, yellowing, and deformed leaves. No clear correlation is evidence of symptoms observed in relation to viruses.

A mixed infection of different viruses in a single plant of cucurbits is also evident (Barbosa *et el.,* 2016; Mutasa *et al.,* 2003). It has reported mixed infection of snake gourd with CMV, CGMMV and PRSV in Sri Lanka by Ariyarathne *et al.,* (2005). CMV, PRSV and ZYMV were found mixed infections in cucurbits in Turkey (Serkan and Filiz, 2013). Mixed infections in cucurbits are frequently observed in natural conditions between viruses from the pot virus genus and CMV, which significantly decreases productivity. These viral species can be naturally transmitted by the same aphid species in a non-persistence manner (Barbosa *et al.,* 2016). Malik *et al.,* (2010) reported that mixed virus infections in plants can increase the severity of the disease symptoms, significantly increasing crop production losses. CMV has the largest known host range. It can also synergistically interact with viruses from the genes potyvirus, increase symptoms and damage to the crop (Chol *et al.,* 2002). However, It has observed several combinations of mixed infection i.e. CMV+PRSV, CMV+PRSV+TRSV, CGMMV+PRSV and CGMMV+PRSV+TRSV with cucumbers, CMV+TRSV with bitter gourd and CMV+CGMMV+TRSV with luffa in present study (Table 2). TRSV is the type species of the genus nepo virus and transmitted by nematodes, varroa mites and honey bees. TRSV is also easily transmitted by sap inoculation and transmission in seeds (John *et al.,* 2014). CGMMV is seed-borne in cucumber with transmission rates reaching 8% and can be mechanically transmitted (Herve *et al.,* 2012). Therefore, It can be interacted TRSV and CGMMV with other viruses associated with cucurbitaceous crops.

As shown in table 2, many samples with virus like symptoms did not record positive reaction for respective virus assayed. This suggests that the samples may have been infested with some other viruses except the particular virus tested. Since cucurbits can be infected test samples with more than 35 viruses (Mahmet *et al.*, 2006), there is a great chance of having infected test samples with other viruses for which samples were not assayed through ELISA. On the other hand, virus like symptoms can be developed due to high infestation of sucking insects and mites (personal communication with extension officers).

Virus like symptoms including leaf mottling, leaf mosaic, leaf distortion, leaf curling, reduce leaf size, resetting and stunting have been reported in major cucurbit crops grown in Sri Lanka such as bitter gourd, pumpkin, Luffa, water melon, snake gourd, cucumber and gherkin. Symptomatology is an important criterion for description of a virus infected crop plant. Many symptoms observed are not virus or crop specific. Therefore symptomology is usually not suitable for diagnosis of a virus disease or identification of a virus.

4. Conclusion

In this study, CMV was the most prevalent virus found in pumpkin, luffa and cucumber. Furthermore, PRSV was the most frequently found virus particularly in bitter gourd. However, ZYMV, ToRSV and CGMMV are not much important virus in cucurbits at present in Sri Lanka. Mixed infection of CMV, PRSV, CGMMV and TRSV is mainly found in cucumbers. Most of the tested plant samples did not react with any tested antisera, yet they displayed virus like symptoms. Therefore, more viruses or virus like agents than the six viruses tested in this study seem to infect cucurbits in Sri Lanka.

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