

***Cucumber mosaic virus* Causal Pathogen of Oily Spots on Cucumber cv. Locale Fruits in Mauritius – Short Communication**

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Abstract

LOBIN K.K., SVOBODA J., LEBEDA A., DHOOKY D.Y., BENIMADHU S.P. (2015): *Cucumber mosaic virus* causal pathogen of oily spots on cucumber cv. Locale fruits in Mauritius – short communication. Plant Protect. Sci., 51: 123–126.

A field survey was carried out in cucumber plantations from different localities in Mauritius to identify the causal pathogen and assess incidence of oily spots on cucumber fruits cv. Locale. ELISA tests on symptomatic fruits showed the presence of CMV and ZYMV. The viruses from the genus *Potyvirus* PRSV and WMV-2 were not detected in the samples screened. Three types of symptoms were observed on infected cucumber fruits: distortion; distortion with oily spots, and oily spots. CMV was detected on fruits showing distortion with oily spots and those with oily spots only. ZYMV was detected in all fruits with distortion and not in those with oily spots only. The above findings showed that oily spots on fruits are caused by infection with CMV. Mechanical transmission of CMV from the oily spotted cucumber fruits to young cucumber cv. Locale plants clearly indicate the association of CMV infection with the oily spot symptoms.

Keywords: CMV; *Cucumis sativus*; field survey; fruit distortion; DAS-ELISA; mechanical transmission

Cucumber (*Cucumis sativus* L.) is a member of the Cucurbitaceae family and is grown in many countries of the world (LEBEDA *et al.* 2007). It is an important food crop grown in Mauritius with an annual production of around 5430 t on 410 ha. Traditionally cucumbers are cultivated in open field from locally available seeds produced by growers and the Ministry of Agro-Industry. The main variety cultivated in open field is the Locale white variety and recently hybrid varieties of white cucumber have also been introduced. Cucumbers are infected by several fun-

gal, bacterial, and viral diseases (ZITTER *et al.* 1996; WALTER *et al.* 2003; HERRERA-VASQUEZ *et al.* 2013). Cucurbit viruses are considered as very important worldwide (LEBEDA *et al.* 1996, 2007).

Virus infection is one of the main causes of diseases of cucumber plants and major viruses infecting cucumber plantation in Mauritius include *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* (PRSV), and *Watermelon mosaic virus-2* (WMV-2) (DOSSA & MUNGUR 1982; ANONYMOUS 1999). Viral diseases of cucumber

Ales Lebeda's part of the research was supported by IAEAVienna, Austria, by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6198959215, and by the Palacký University in Olomouc Internal Grant IGA_PrF_2015_001. Jiří Svoboda's part was supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE RO0414.

doi: 10.17221/9/2015-PPS

plants result in yield losses through reduction in growth and are responsible for distortion and mottling of fruits, making the product unmarketable. A virus infection on cucurbits in Mauritius was first reported in 1978 causing leaf distortion and stunted growth (ANONYMOUS 1978). CMV is one of the most important viruses infecting cucumbers and aphids are the main and most efficient vectors of the virus (ZITTER & BANIK 1984). More than 60 species of aphid, notably *Aphis gossypii* and *Myzus persicae*, can transmit CMV in a non-persistent manner, and the virus is readily transmissible through the infected plant sap (FRANCKI *et al.* 1979). Several strains of the virus have been registered (BOLTON & NUTTALL 1971; ROOSSINCK 2002). Symptoms consist of downward cupping along the midvein and leaf reduction from which the plants fail to recover. CMV can cause fruit deformation and mottling on fruits (ZITTER & MURPHY 2009).

Since 2005, wart (oily spot) symptoms have been commonly observed on cucumber fruits of the white variety Locale grown on the island widely. In severe infections, the spots cover the whole surface of the fruit causing it to be unmarketable. A survey was carried out in 2006 and 2007 to assess the incidence of viral infections on cucumber plantations in Mauritius and to determine the cause of oily spot symptoms on cucumber fruits.

MATERIAL AND METHODS

Field survey. In 2006 and 2007, field visits were carried out to assess virus distribution in cucumber plantations all around the island. Symptomatic leaf and fruit samples were collected for investigating the etiology of the oily spot symptoms. Fruits were collected from cucumber vines exhibiting viral symptoms such as leaf mottling, distortion, blisters, and stunted growth. Three types of symptoms were observed on fruits: distortion, distortion with oily spots and oily spots. Based on symptoms expressed, the collected samples were screened for the presence of viruses from Potyvirus genus and CMV.

ELISA. DAS-ELISA (CLARK & ADAMS 1977) was used for the screening of the collected symptomatic samples. Commercially available CMV, PRSV, WMV-2, and ZYMV rabbit polyclonal antibodies (Loewe Biochemica, Sauerlach, Germany) were used according to the manufacturer's manual. Samples for ELISA were prepared by grinding 0.3 g of plant tissue in phosphate

buffered saline (pH 7.4) with 2% polyvinylpyrrolidone and 0.2% of bovine albumin in the ratio 1 : 20. Positive and negative controls were included on each ELISA microtiter plate to improve the validity of the tests. The plates were incubated for 1–2 h at 20°C after pipetting the substrate (*p*-nitrophenyl phosphate) solution, and the absorbance value was read at 405 nm using a MR 5000 Dynatech reader (Dynex Technologies GmbH, Denkendorf, Germany). A reaction was considered positive when the absorbance value was at least five times higher than that for the negative control.

Mechanical transmission. A thin skin sample of the cucumber fruit positive in ELISA for CMV was used for a mechanical inoculation. Inoculum was prepared by homogenising 1 g of the sample with 3 ml of 0.07 M Na₂HPO₄, pH 7.0. Carborundum (200 mesh) was added to the homogenate and young cucumber cv. Locale or summer squash cv. Zelena (*Cucurbita pepo* L. convar. *giromontiina* Grebenščíkov) leaves were then inoculated by friction them by fingers in gloves wetted in the inoculum. The plants prepared for inoculation were kept for 24 h before the inoculation in darkness to improve the transmission efficiency. After inoculation, the plants were sprayed with distilled water and grown in a greenhouse under a light and dark photoperiod of 14 and 10 h at 25 and 20°C, respectively.

Pathogenicity tests. The tests were performed to establish a link between formation of oily spots on cucumber cv. Locale fruits and a virus presence in plants. The inoculum prepared from collected oily spot fruits was used on young cucumber seedlings at 2–3 true leaves stage in a greenhouse. Inoculation was done mechanically on ten developing seedlings transplanted in plastic pots. A control was set up with ten non-inoculated seedlings. The seedlings were kept in a greenhouse under insect proof condition.

RESULTS AND DISCUSSION

In 2006 and 2007, an etiological study of viral diseases was carried out in cucumber plantations in Mauritius. Symptomatic plants showed leaf drying at its base, interveinal yellowing, mosaics on new growths and wart (oily spot) symptoms (Figure 1) on their fruits. Symptoms of oily spots were observed on fruits at different developmental stages from small developing fruits to mature fruits and the severity was low (few spots but fruit still marketable) to severe (covering the whole fruit surface and unmarketable).

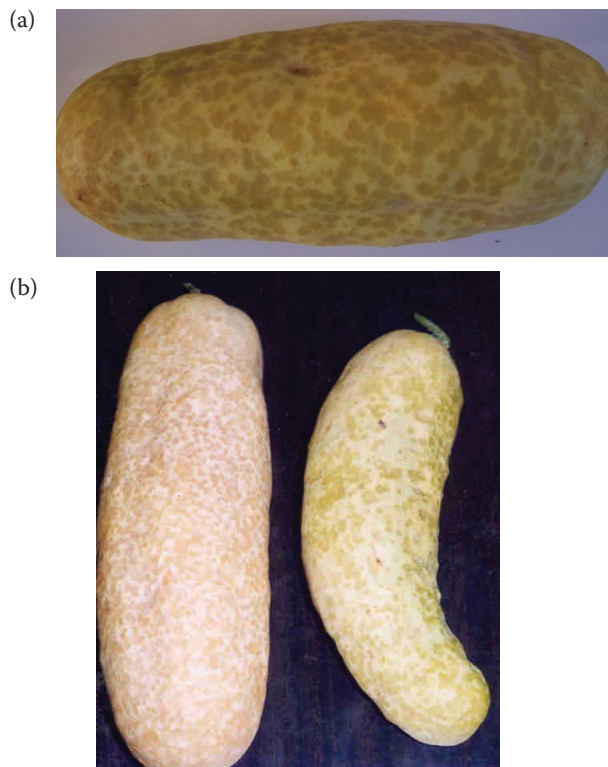


Figure 1. Cucumber fruits of Locale variety showing (a) oily spot symptoms and (b) oily spots with distortions (Photo K.K. Lobin)

Severity did not depend on the developmental stage of plants, however it depended on fields. Percentage incidence of oily spots on fruits in the field varied and was up to 100% infection.

During the survey, a total number of 177 cucumber fruits were collected from symptomatic cucumber vines across the island and screened for the presence of CMV, ZYMV, PRSV, and WMV-2 by ELISA. The collected symptomatic fruits showed three types of symptoms: distortion, distortion with oily spots, and oily spots. 35 fruits (19.8%) with distortion symptom only were positive to ZYMV, 50 fruits (28.2%) with distortion and oily spots symptoms were positive to both ZYMV and CMV (mixed infection), and 92 fruits (52%) with oily spot symptoms were positive to CMV. All the collected fruits were negative to PRSV and WMV-2.

Twenty-one collected fruits which were positive in ELISA only to CMV and with evident oily spots were selected to confirm the involvement of CMV in oily spot formation. An inoculum was prepared from the spotted fruits and the mechanical transmission on ten young cucumber cv. Locale seedlings was performed in a greenhouse. Mottling symptoms appeared on inoculated plants (alternatively: chlorotic local lesions appeared on inoculated leaves 4–6 days

post inoculation and systemic symptoms – mosaic or mottling – expressed) leaves 2 weeks after the inoculation whilst no symptoms were observed on non-inoculated control plants. Hand pollination was carried out at the flowering stage to allow fruit formation. The infected plants produced fruits with oily spot symptoms. Fruits without symptoms were produced from the non-inoculated control plants. All of the ten inoculated plants expressed virus symptoms and none of the ten control plants were symptomatic. ELISA tests carried out on symptomatic leaves from inoculated plants confirmed the infection by CMV. The above findings clearly show that oily spots on fruits are attributed to the infection with CMV.

Cucumber fruits harvested from the mechanically inoculated plants were used to perform the parallel test for virus presence at the Crop Research Institute, Prague, Czech Republic. Altogether four samples were taken from the examined cucumber fruits, two from the skin and two from the interior. CMV was detected by ELISA in all the samples with absorbance value higher than 1.0 compared to the absorbance value of the negative control less than or equal to 0.02. Tests for the presence of WMV-2 and ZYMV were negative. CMV was further confirmed by the mechanical transmission of the virus on four indicator summer squash cv. Zelena plants. After ten days, all the indicator plants were stunted and showed severe symptoms as yellowing and deformation of their leaves with formation of chlorotic local lesions well known for the presence of CMV (Plant Viruses Online 2013). The CMV infected summer squash leaf sample was deposited in the Virus Collection of Crop Research Institute, Prague, Czech Republic (Virus Collection 2013).

To conclude, this study showed the association of CMV on oily spots of cucumber cv. Locale fruits in Mauritius. The results indicate that the single infection of CMV causes oily spots on cucumber fruits. Mixed infection with ZYMV has also been detected. It causes abnormal distortions on fruits together with oily spots.

Oily spot of cucumber is a serious disease causing economic losses in Mauritius. The disease is spread islandwide in cv. Locale fruits, white variety of cucumber grown in the open field. No such symptoms have been observed in greenhouse grown cucumber varieties. As the oily spot symptoms have been observed on cucumber fruits in Mauritius since 2005, it could be concluded that it is a relatively new disease of increasing importance.

CMV has a wide host range, infecting more than 1200 plant species in 100 families (EDWARDSON

doi: 10.17221/9/2015-PPS

& CHRISTIE 1991). The virus has been reported to be seed-borne in pepper (ALI & KOBAYANSHI 2010). Similarly TÓBIÁS *et al.* (2008) have reported on the seed-borne nature of ZYMV and CMV in the cucurbit *Cucurbita pepo*. In the view of the economic importance of the disease, an epidemiological study should follow to investigate on the seed borne aspect of the CMV on cucumber in Mauritius and also on the distribution of aphids which are as vector causing its spread in the field. These studies should advise appropriate management practices reducing the impact of the disease on cucumber in Mauritius.

It would be reasonable to sequence the severe CMV strain as a possible new strain causing the disease and compare the obtained sequences to the GenBank data. Another mild CMV strain may be helpful in cross-protection cucumbers against the severe one in Mauritius.

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Received February 5, 2015

Accepted after corrections March 10, 2015

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