EFFECT OF PEPPER MILD MOTTLE VIRUS INFECTION ON PEPPER AND TOMATO PLANTS

Nikolay Petrov
Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov"
Administration: 1331 Sofia, 7 Shosse Bankya Str., Bulgaria
Department of Plant Protection: 2230 Kostinbrod, 35 Panayot Volov Str., Bulgaria
m_niki@abv.bg

ABSTRACT

Pepper mild mottle virus (PMMoV) belongs to the genus Tobamovirus. It is spread in different soil areas and abundant in wastewater showing the ability of this virus from year to year, gradually to increase its range of distribution and to be an indicator of fecal pollution. Despite the presence of PMMoV in human feces, this virus was not detected in the majority of animal fecal samples tested, with the exception of chicken and seagull samples. The virus is very resistant to the environmental conditions and at the same time is a threat to its distribution in crops of tomato and pepper. It is spread by mechanical transmission and infected seeds. Virus symptoms includes various degrees of mottling, mosaic, chlorosis, necrosis, curling, dwarfing, and distortion of the fruits, leaves, and even whole plants. The symptoms on fruit include: a reduction in size, mottling and color changes and it frequently results in significant crop losses or reductions in both field and greenhouse plantings. The symptoms are far more pronounced if the plants were infected when they were young rather than when they were older. This disease is harmful because of the mild foliar symptoms and due to this many times the pathogen goes unnoticed until the more evident symptoms on the fruit appear. This is why there are higher yield losses because symptoms only become evident during the fruiting stage right before the crops are supposed to harvest. Virological testing of the plants at an early stage would limit losses to producers.

***Key words**: PMMV, pepper, tomato*

Introduction

Pepper mild mottle virus (PMMoV) was first described in Italy in 1984. Since then, it has spread and become a significant pathogen of pepper crops worldwide (Wetter, 1984). PMMoV is a member of the genus *Tobamovirus*, with a positive-sense genomic RNA of 6,400 nucleotides in length encoding at least four proteins: 130K and 180K replication proteins, a movement protein, and a coat protein (CP) (Fauquet, 2005).

PMMoV infects cultivated pepper plants through seed and soil transmission in the fields, causing severe mosaic symptoms. PMMV is not transmitted by insects. It can be seedborne, consequently, the seedlings can be infected by mechanical contamination from their seed coats during transplanting or other cultural procedures. This is a primary source of infection. Foliar symptoms of PMMV consist of mottling and yellow/green mosaic, while fruit may be small, malformed and mottled, with sunken or raised necrotic spots. Yield loss is considerable when young plants become infected. When the virus contaminates once in a green pepper field by carrying over seed, it is extremely difficult to get rid of it. The virus in infected plants remains as long as green pepper is cultivated continuously in that field, because the plants later becomes sources to infect newly transplanted young seedlings (Agrios, 2005).

The virus is quite stable and highly infectious and is easily spread from plant to plant during normal crop maintenance. Also, the virus can persist in the previous crop in infected pepper debris such as leaves, stems or roots in soil for several months.

Methyl bromide is the only chemical to effectively prevent virus spread by fumigation in soil, and has been used between cultivations in fields (Yoneyama, 1988). Recently, however, an international decision (the Montreal Protocol) determined that methyl bromide was no longer to be used after 2005 in developed countries, due to environmental politics. Consequently, alternative strategies to control virus disease will be required as soon as possible for green pepper fields infested with PMMoV. One effective strategy to protect pepper from PMMoV damage is the use of an attenuated virus as a plant virus vaccine. Although some attenuated PMMoV strains were created previously in Japan, they still caused mottle or mild mosaic symptoms when used in pepper fields (Goto, 1997; Goto, 1984; Nagai, 1987).

PMMoV is a non-enveloped, rod-shaped, single stranded positive sense RNA virus, extremely resistant to physical and chemical agents (Wetter, 1984). It is one of the major pathogens of Capsicum spp (chili peppers). PMMoV could be detected in nondiarrheic stool from 18 individuals living in San Diego, USA or in Singapore, suggesting it might be geographically widespread, and in 3 out of 22 fresh and processed pepper samples. Moreover, the fecal PMMoV was viable and could infect host plants (Colson, 2010). PMMoV RNA sequences were recovered from twelve (57%) of the twenty-one pepper- or spice-containing food products. Tabasco sauce contained the highest viral load, estimated to be nearly 107 PMMoV RNA copies/ml based on a cycle threshold of 22 by real-time PCR or the presence of more than one viral particle/field by electron microscopy (Colson, 2010). Humans may carry a high PMMoV load, likely acquired from food products, and was proven that PMMoV might not only be a common inhabitant of the human gut but may also interact with the human immune system and cause clinical symptoms. These results should prompt further studies to re-evaluate whether or not plant viruses, including PMMoV, may have a pathogenic role in humans (Colson, 2010).

Material and methods

Tomato cultivars used: cv. Ideal, cv. Naslada, cv. Stryama, cv. Rila, cv. Kalina, cv. Buffalo heart, cv. Cherry, cv. Bononia, cv. Mila, cv. Heart of the Albeng.

Pepper cultivars used: cv. Kurtovska kapia, cv. Red kapia, cv. Septemvriyska kapia, cv. Amfora, cv. Elephant ear.

DAS-ELISA (Double Antibody Sandwich Enzyme Linked Immunosorbent Assay):

The analysis was conducted by the method of Clark and Adams (1977). We used a commercial kit of LOEWE Biochemica GmbH, Sauerlach, Germany. ELISA plates were loaded with antiserum (IgG) for PMMV, with dilutions (according to the instructions of the manufacturer) in 0.05 M carbonate buffer. The samples were incubated for 4 hours at 37 ° C, and the unbound components were washed out with PBS-T buffer for 5 min. All samples were grounded in extraction buffer containing 1% PVP (polyvinyl pyrrolidone) in a ratio of 1:10. The plates were incubated at 4 °C for 16 hours. Following the third wash step alkaline-phosphatase conjugate for PVY was added and the plates were incubated for 4 hours at 37 °C. The used substrate was p-nitrophenyl phosphate (p-nitrophenyl phosphate, Sigma) in diethanolamine buffer (pH 9.8) at a ratio of 1mg/ml. The reaction proceeded in the light at room temperature and was stopped with 3N NaOH. The adsorption of the color reaction was measured at multifunctional detector (DTX 880) at a wavelength of 405nm.

The positive samples had optical density (OD) over the threshold (Cut-off) which was two times the value of the negative control.

Results and discussion

Symptoms on the diseased plants:

Virus symptoms includes various degrees of mottling, mosaic (Fig. 1), chlorosis, necrosis, curling, dwarfing, and distortion of the fruits, leaves, and even whole plants. The symptoms on fruit

include: a reduction in size, necrotic spots (Fig. 2), mottling and color changes (Fig. 3), necrotic patterns (Fig. 4) to severe necrosis of the fruit (Fig.5), and it frequently results in significant crop losses or reductions in both field and greenhouse plantings. The symptoms are far more pronounced if the plants were infected when they were young rather than when they were older. This disease is harmful because of the mild foliar symptoms and due to this many times the pathogen goes unnoticed until the more evident symptoms on the fruit appear.

Fig. 1 PMMV leaf mosaic

Fig.2 PMMV necrotic fruit spots

Fig.3 PMMV fruit discoloration

Fig.4 PMMV fruit necrotic patterns

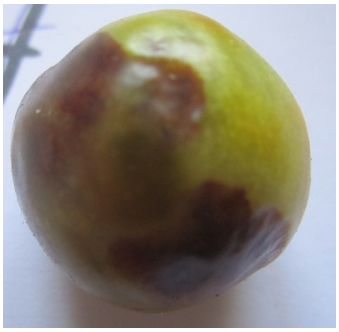

Fig.5 PMMV severe necrosis

Samples from ten tomato cultivars and five pepper cultivars were tested with DAS-ELISA for the presence of PMMV. The result indicate that only one tomato cultivar cv. Stryama and two pepper cultivars cv. Kurtovska kapia and cv. Amfora was infected with PMMV (Fig.6).

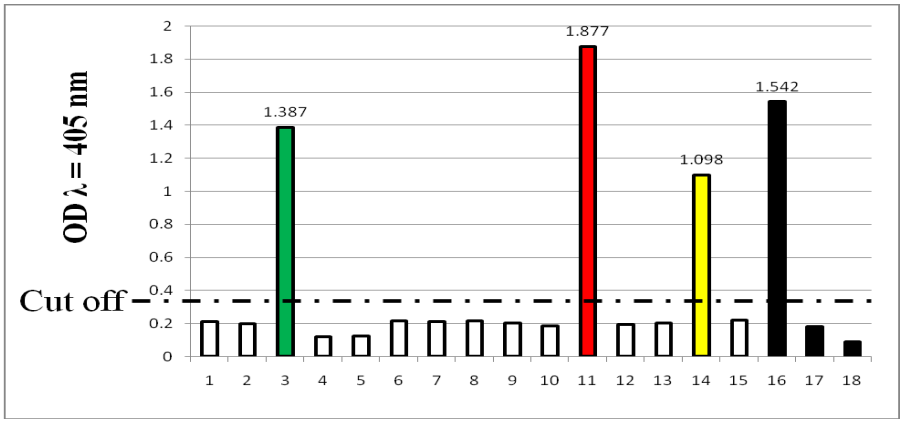

Fig.6 DAS-ELISA from tomato and pepper cultivars

Conclusion

There are higher yield losses because symptoms only become evident during the fruiting stage right before the crops are supposed to harvest. Virus testing of the plants at an early stage would limit losses to producers and stop wide spreading of the virus to other plants in the crop.

References

1. Agrios, G. N. 2005. Plant Pathology. Fifth Edition. Elsevier Academic Press, London.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme - linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.
3. Colson, P., Richet, H., Desnues, C., Balique, F., Moal, V., Grob, J., Berbis, P., Lecoq, H., Harle´, J., Berland, Y., Raoult, D. 2010. Pepper Mild Mottle Virus, a plant virus associated with specific immune responses, fever, abdominal pains, and pruritus in humans. *PLoS ONE*, Vol 5 (4), 10041- 10041
4. Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., and Ball, L. A. 2005. Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London.
5. Goto, H., Itai, T., and Sato, S. 1997. Selection of attenuated viruses for control of sweet pepper mosaic disease caused by *Tobacco mosaic virus* pepper strain and Cucumber mosaic virus, and their effects. Bull. Oita Prefect. Agric. Res. Cent. 27:79-122.
6. Goto, T. 1984. Utilization of attenuated strain for control of pepper mosaic disease caused by tobacco mosaic virus. Crop Prot. 38:349-352.
7. Nagai, Y. 1987. Production of C-1421, an attenuated mutant of pepper strain of tobacco mosaic virus. Ann. Phytopathol. Soc. Jpn. 53:168-174.
8. Wetter C, Conti M, Altschuh D, Tabillion R, van Regenmortel MHV (1984) Pepper Mild Mottle Virus, a Tobamovirus infecting pepper cultivars in Sicily. Phytopathology 74(4): 405–10.
9. Wetter, C., Conti, M., Altschuh, D., Tabillion, R., and van Regenmortel, M. H. V. 1984. Pepper mild mottle virus a tobamovirus infecting pepper cultivars in Sicily. Phytopathology 74:405-410.
10. Yoneyama, S. 1988. Control of P strain of tobacco mosaic virus in sweet pepper. (3) Effects of soil fumigation with methyl bromide in summer season. Kanto-Tosan Plant Prot. Soc. 35:56-57.