To: Mr. Ofer Nidam, DUSMIT.

Hereby attached the test repot:

"Examining the ability of DUSMIT system to remove virus particles that were artificially aerosolized using a Bio- Assay".

Principle

Detection of infectious *tobamoviruses* by inoculating leaves of indicator plants *Nicotiana tabacum* cv. *Xanthi* NN and *Nicotiana glutinosa*. Infectious virions cause typical local lesions that demonstrate viability of the virus.

In plant pathology, estimating the quantities of infectious virus present in different preparations is of great importance. The Bio - Assay method, developed by Holmes in 1929[1], is a basic technique for determining the relative infectivity. Upon mechanical inoculation of leaves with a virus suspension, the number of resulting infected sites is dependent on the virus concentration in the inoculum. If the infected sites appear as discrete spots (local lesions), they can be counted. Their numbers may allow conclusions as to the amount of infectious virus in the sample. However, virus concentrations determined in an infectivity assay are never absolute, but only relative, as not all virus particles are infectious and not all cells are accessible to the virus particles, probably due to the inefficient wounding of the outer cell walls during inoculation and inadequate introduction of virus into the cells.

Materials and Methods

1. Four tobacco plants, two Nicotiana tabacum cv. Xanthi NN and two Nicotiana glutinosa were dusted with carborundum powder (SiC) until a very fine layer covered the leaf surface.
2. A drop of Phosphate Buffered Saline smeared on all plants' leaves with gloved fingers by applying constant, slight pressure in order to wound the leaves.
3. Plants were placed in a 2 plastic chambers (see pictures) before the DUSMIT system and at the end of the system.
4. One *Nicotiana tabacum cv. Xanthi* NN and *one Nicotiana glutinosa* at the first chamber, at the beginning of the DUSMIT system and *one Nicotiana tabacum cv. Xanthi* NN and one *Nicotiana glutinosa* at the second camber, at the end of the system.
5. Chambers were sealed and the virus suspension was sonicated in the first chamber, until the chamber was full of fog.
6. After one minute, the air was vacuumed into the DUSMIT system in four rounds.
7. Next, it was steamed into the second chamber.
8. In the second chamber, after it was completely full with the vacuumed air from the first chamber, the air was paused for one more minute.

9. Following one minute, the air was vacuumed into an autoclaving system (121°c 20min) and out to the open air.
10. The boxes were transferred to a controlled green house (25±3°C) for 7 days.
11. After 7 days we counted the number of local lesions developed on each leaf in all plants. Results presented in the table below.
12. Moreover, after 7 days, in order to look for virus residues in the boxes, we sampled the sides of the two boxes by thorough wiping and inoculating one plant of *Nicotiana tabacum cv. Xanthi* NN from each box, using the same method (control).
13. Plants were transferred to a controlled green house (25±3°C) for 7 days and symptoms were counted.

Results

Table 1: Number of local lesions on plants leaves located before the DUSMIT system (Box 1)

Leaf number	Day 0		Day 7		Box's sides Day 7
	Nicotiana Xanthi	*Nicotiana Glutinosa*	*Nicotiana Xanthi*	*Nicotiana Glutinosa*	*Nicotiana Xanthi*
1	0	0	2	0	0
2	0	0	1	0	0
3	0	0	3	0	0
4	0	0	2	0	0
5	0	0	1	1	0
6	0	0	2	0	0
Mean	0	0	1.83	0.17	0

Table 2: Number of local lesions on plants leaves located at the end of DUSMIT system (Box 2)

Leaf number	Day 0		Day 7		Box's sides Day 7
	Nicotiana Xanthi	*Nicotiana Glutinosa*	*Nicotiana Xanthi*	*Nicotiana Glutinosa*	*Nicotiana Xanthi*
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
Mean	0	0	0	0	0

Conclusion

1. The method was fit for purpose.

2. We succeeded to inoculate the plants using the Bioassay method.

3. Infectious *tobamoviruses* created local lesions on tobacco leaves at the first box, placed before the DUSMIT system.

4. However, no local lesions developed on tobacco leaves at the second box, placed at the end of DUSMIT system.

5. We can assume there were no viable virus particles at the second box, after passing through the DUSMIT system.

Sincerely,

Atalya Keshet - Sitton Ph.D

Head of Biology Lab, Bactochem Laboratories LTD.

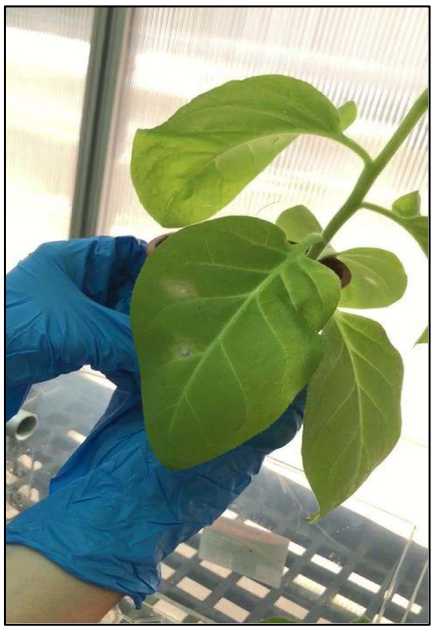
References

Scholthof, K.-B.G.. (2011). TMV in 1930: Francis O. Holmes and the local lesion assay. Microbe. 6. 221-225.

ISTA rules for seed testing (2020). 7-028: Detection of infectious tobamoviruses on Solanum lycopersicum (tomato) by the local lesion assay (indexing) on Nicotiana tabacum plants.

Appendix

Mrs. Veronika, our Lab technician, applies carborundum powder (SiC) on *Xanthi* leaves prior to inoculation with an aerosol of *tobamoviruses* (**Day 0**)



Local lesions developed on *Xanthi* plants, following inoculation with an aerosol of *tobamoviruses*. Plants were placed **before** the **DUSMIT system** (**Day 7**).

Xanthi plants with **NO** local lesions. Plants were placed **after** the **DUSMIT system** (**Day 7**). Therefore, we can assume that the aerosol containing virus particles, which passed through the DUSMIT system and then entered the second chamber, was free of viable