Lab2

Virus cultivation and propagation In Plant Viruses

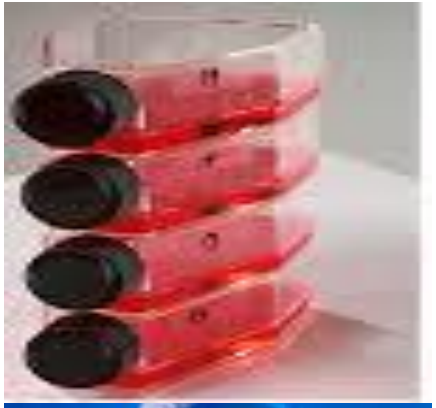


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VIRUS CULTIVATION AND PROPAGATION

- Virus cultivation is also referred to as propagation or growth.
- Since viruses are obligate intracellular parasites, they **cannot** be propagated in any cell-free medium, regardless of its composition, and must be grown **within living cells.**
- Virus cultivation involves growing viruses either **in vitro** (outside a living host in cell cultures, eggs, or tissues) or **in vivo** (inside a whole living organism - host- :animal or plant)
- Following infection, pathological effects appear in cultured cells or in the infected organism, and the virus may cause **complete lysis and death** of the infected cells.









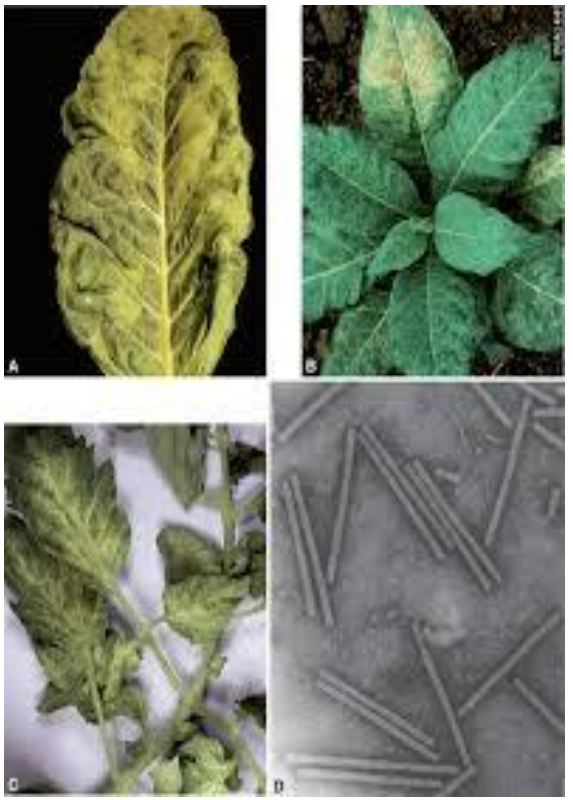
Virus cultivation and propagation In Plant Viruses

- Plant viruses may be propagated **in vitro** by infecting cell wall–free plant cells (**protoplasts**) or **in vivo** by infecting **suitable plant hosts** to assess viral pathogenicity.
- Young plants maintained under **insect-free greenhouse** conditions are commonly used.
- Certain plant viruses require **biological vectors**, including insects or nematodes, for transmission; these vectors are maintained in the laboratory, allowed to acquire the virus from infected plants or viral extracts, and subsequently transferred to healthy plants in a process referred to as **biological infection.**



- To quantify infectious TMV particles in plants, **Francis Holmes** introduced a **lesion-counting assay** in which localized necrotic lesions serve as indicators of successful viral infection.
- Due to the absence of viral receptors and the presence of rigid cell walls in plant cells, viral entry requires **mechanical inoculation** facilitated by abrasives and the application of purified virus preparations or infected plant sap.





MECHANICAL INOCULATION

Materials:

Infected tobacco plants by TMV , pestle and mortars, muslin cloth, glass beakers, test tubes, host plant.

Capsicum annuum الفلفل **35**

Lycopersicon esculentum الطماطم **28**





MECHANICAL INOCULATION

Procedure:

There are four steps

a. Plant sap extraction.

b. Preparing the suitable host for mechanical inoculation.

c. Performing the mechanical inoculation

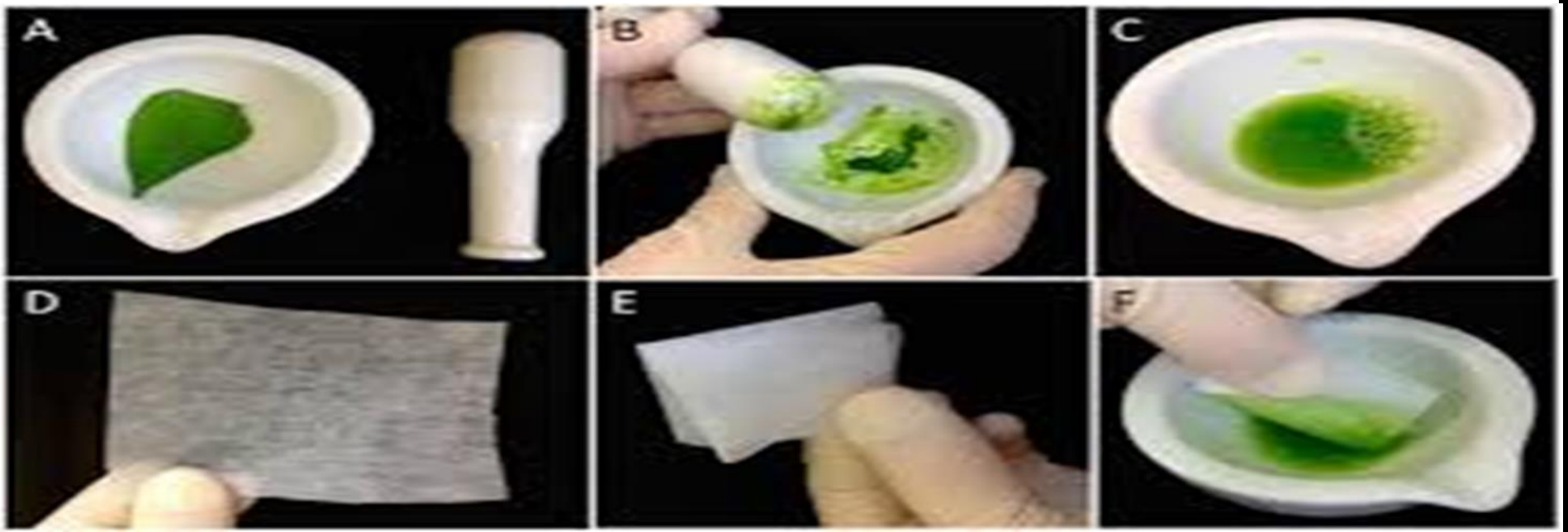
d. Keeping the plants in the greenhouse

Before performing the mechanical inoculation, the information must be written on the plant pot: (Group name, inoculation date).

1.Prepare the plant sap by grinding virus- infected leaves in sterilized pestle and mortar. In such case phosphate buffer is mixed after proper grinding.

(1 gram of virus-infected leaves to 5 ml of buffer solution).

2.After thorough grinding the whole leaves pulp are strained through a piece of muslin cloth to get filtered extract of the leaves and collect it in beaker.



3.The leaves of test plants are washed properly and gently blotted dry with tissue/blotting paper.

4.On the leaves of the test plants, carborundum powder is lightly dusted to cause injury on the leaves to be inoculated.

5.A piece of muslin cloth soaked in such extract is rubbed on the leaves of test plants , in one direction only from the petiole on the apex of the leaf.



6.After inoculation, inoculated leaves are Sprayed with water in order to remove excessive inoculate and carborundum powder and to re-swell the cells.

7.Inoculated plants are kept in the glass house for observation, their watering and the onset of symptoms periodically



ILLUSTRATION OF MECHANICAL INOCULATION OF PLANT VIRUSES

