PLANT TISSUE CULTURE .LAB (4)

# Sterilization

(THE FOURTH STAGE)

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# Sterilization

- The sterilization process in a tissue culture laboratory consists of three main parts
- **1. sterilize media**:- and are using Autoclave device and have a temperature of 121 C° pressure 1.04 kg / $cm^2$ for 20 minutes.
- **2. sterilize the tools**:- (forceps and blades and dishes etc ...)It can be sterilized by autoclaving after are wrapped in aluminum foil and placed in the oven on a 100C° degree heat for at least an hour before use.
- **3. sterilize parts planted**: - Either: - using distilled water alone like Apical meristem.
  - Or : - using ethanol after being washed with distilled water and ethanol have a concentration of 96% with stirring for ± 2 minutes
  - Or : - washed with Sodium hypochlorite (minor) NaOcl after being washed with distilled water with certain concentration for a period of approximately 10 minutes, then wash again diameter sterile water three times to remove traces of Article sterile
  - Or : - sterilized ethanol for a certain period and then washed with sterile distilled water and then sterilized with Sodium hypochlorite for a certain period and then washed with sterile distilled water 3 times
  - Or : - washed with distilled water and then sterilized with Mercuric chloride concentration of 0.01% for a certain period rather than NaOcl

# (MS) Stocks and Media Preparation

- This step involves the preparation of Stocks of various media components (previously mentioned) and stored at 4-5°C until used in preparing the required medium. Media preparation is done in the following mode:-

- 1- It weighs 20-30 g sucrose or as desired.
- 2- Weigh 100 mg of inositol.
- 3- The major elements are weighed as shown in the media table.
- 4- 1ml of each of the three previous solutions for micronutrients are taken and vitamins are added as shown in the media table.
- 5- Hormones are placed according to the type of hormone.
- 6- Complete the volume to a liter (1000 cm 3) distilled water.
- 7- The pH is set to be 5.6 to 5.8 solid and from 5.2 to 5.5 liquid.

- 8- Agar is placed for the solidification of the media, which is about 7g /L. This depends on the type of agar and sometimes it is liquid without agar and the media is cooking on a magnetic stirrer heater until all these ingredients are mixed.
- 9- The environment shall be applied to jars or test tubes about 10 cm for the tube and then covered with special covers made of polypropylene, which shall bear the pressure and temperature at sterilization.
- 10- Sterilization of pipes or jars in autoclave at 121°C and air pressure 1.04 kg/cm² for 20 minutes.
- 11- Cool the jars and tubes containing the media and put the pipe tilted in special ways at angle of 45 degrees to agriculture.

## A quick summary of the key points:-

- 1- Standard autoclave parameters: temperature of 121 C°,pressure 1.04 kg/cm², for 20 minutes.
- 2- Surface sterilization of plant material: Methods vary—using distilled water, ethanol (96\%), sodium hypochlorite, or mercuric chloride (0.01\%)—depending on the nature of the plant part.
- 3- Preparation of MS medium: This begins by mixing macronutrients, micronutrients, vitamins, hormones, sucrose, and inositol; followed by adjusting the pH, adding agar, sterilizing, and finally cooling and pouring the medium.