Sterilization of Plant Material

Tissue cultures must be carried out under aseptic conditions. Consequently plant materials must be sterilized, to get rid of any adhering microorganisms, before placing on tissue culture media. Before sterilization, plant material may be washed in soapy water to remove soil particles and dust and to enhance the contact of the disinfectant. Plant materials are then surface sterilized in disinfectant solution to which a wetting agent (eg: Tween-20) is added. Wetting agent acts as surfactant. After shaking for several minutes, explants are rinsed aseptically in sterile distilled water. Several disinfecting agents are used eg: commercial bleach (5.25% sodium hypochlorite), calcium hypochlorite, ethanol (70%), hydrogen peroxide, mercuric chloride and silver nitrate. Disinfectants also have lethal effects on tissues of explant. Consequently, the concentration of disinfectant and time of treatment are manipulated to achieve maximum percentage of contamination-free viable cultures. Usually two disinfectants are used in sequential manner.

Example: Sterilization of Canola Seeds

1- In 250 ml beaker, shake about 2 g canola seeds in 50 ml ethanol (70%) for one minute.
2- Transfer seeds and ethanol to a sieve to get rid of ethanol.
3- In 250 ml glass jar, shake the seeds in 100 ml Clorox solution (30%) for 25 minutes.

In laminar flow cabinet

4- Transfer seeds and Clorox to a sterile sieve to get rid of Clorox.
5- In 250 ml sterile glass jar, shake the seeds in 100 ml sterile distilled water.
6- Repeat the previous step 3-4 times to remove remains of Clorox.
7- Place the seeds on germination medium (10 seeds/jar).
8-Incubate cultures, for 7 days, at 25 °C under cool-white fluorescent light (2000 Lux irradiance) with 16-hour photoperiod.
9- Record Contamination% = (No. of Contaminated Cultures / Total No. of Cultures) X100

Germination % = (No. Germinating Seeds / Total No. of Seeds) X100

The best treatment is that giving the highest number of living (able to germinate) contamination-free seeds.

Q1: You are provided with Commercial bleach (Clorox) containing 5.25% sodium hypochlorite.

Describe preparation of the following concentrations:

1- **45% Clorox**
2- **4% Sodium hypochlorite**

Q2: The following table shows the contamination (shaded cells) and germination of canola seeds following sterilization with different treatments with Clorox. The results were collected from 10 cultures, each contains 10 seeds.

Identify the best treatment for sterilization. Why?

Treatment		**Jar Number**									
Time (min)	**Concn. (%)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
10	**10**	8	9	8	9	7	8	7	8	8	9
	20	7	6	7	8	6	8	8	7	8	8
	30	7	6	5	5	5	7	7	7	6	7
20	**10**	8	8	7	8	6	8	7	6	8	8
	20	5	6	7	6	5	7	6	6	7	8
	30	3	4	4	5	5	6	5	6	7	6
30	**10**	5	4	5	5	3	5	6	5	5	6
	20	1	3	2	2	4	2	2	3	3	3
	30	0	0	0	0	0	0	0	0	0	0