Date:

Name:

Topic: **Seed Disinfections, Sterile Sowing and Germination**

Material: Seeds of various lines of tobacco (*Nicotiana tabacum* L. SR1, *Nicotiana tabacum* cv. Xantha), flax (*Linum usitatissimum* L.), carrot *Caucus carota* L. *ssp. sativus* (Hoffm.) Arcang. cv. Karotela, cv. Nanteská, etc.

Medium: M-S basal salts, B5 vitamins, sucrose 20g.l^{-1}, agar 8g.l^{-1}

Procedure: Seed surface disinfections

1. Place the seeds into a glass tube or tissue bag (depends on the seed size).
2. Disinfections in 50% ethanol and 3% hydrogen oxide for 1 min.
3. Wash in sterile distilled water (SDW).
4. Disinfections in aqueous solution of 20% commercial bleach SAVO (v/v) by agitation for 20 min.
5. Rinse 3x in sterile distilled water for 3 - 5 min. each and place into a sterile beaker for imbibitions in a laminar flow hood.
6. Imbibitions for 24 hours.
7. Repeated disinfections in aqueous solution of 20% commercial bleach SAVO (v/v) for 20 min.
8. Rinse the seeds in sterile distilled water 3x for 3 - 5 min.
9. Transfer of sterilized seeds into a sterile Petri dish with filter paper.

Sterile seed sowing

10. Sterile sowing of sterilized seeds on the surface of agar solidified M-S medium in a Petri dish. Tightly seal the Petri lids with foil or Parafilm to prevent their opening. Label the dish (culture, date, name).
11. Incubate the cultures in culture room in the light (cool white fluorescent lamps) or in the thermostat in the dark at temperature 25°C.
12. Record the composition of media – concentration of inorganic and organic nutrients, agar and sucrose as well as the number of seeds.

EVALUATION:

Evaluate the germination capacity (%) of the seeds in the following weeks of the culture. Monitor the development of seedlings and the expression of sensitivity on the selection media. Control the contamination of the culture. Discard contaminated culture. Use the seedlings in the alternative exercises.