University of Agronomic Sciences and Veterinary Medicine of Bucharest
Faculty of Horticulture

International Student Symposium *"Hortus Academicus"*
December 11, 2020, Bucharest, Romania

research on the *in vitro* propagation of

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COSTMARY

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Tanacetum balsamita L.

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Costmary

Tanacetum balsamita L.

Justification

- *Tanacetum balsamita* L. (Costmary) has recently come into the attention of the scientific community (and the pharmaceutical industry) due to its high content in **special active substances** (volatile oils, bitter substances, tannins, saponins, flavones) - substances with strong **anti-inflammatory, antifungal** and **antibacterial** properties.

Justification

- **Micro-propagation**, through the massive proliferation of a special individual (rich in active substances of interest) is an **economically efficient response** with an extremely productive potential

! in a global market of guaranteed and certified quality of raw materials, these ‘raw materials’ are generating active substances of much better quality, better tolerated by the human body, with a more complex structure, in total harmony with the environment and much cheaper then their chemically synthesized copies

Objectives

- the **main objective** of the present research was to study the *in vitro* multiplication of *Tanacetum balsamita* L., focusing on:
  - the percentage of germination after seed disinfection,
  - the tissue increases in length and volume

  and
  - establishing the most suitable culture medium for multiplication by seeds and by explants.

Objectives

- intermediate / secondary objectives were:
  - documenting the seed germination capacity
  - finding a favorable disinfection protocol
  - identifying a growth-friendly inoculation medium
  - identifying a favorable multiplication medium and multiplication rate

the Venue

- was in the USAMV campus – at the micro-propagation laboratory of CCSCPA “Hortinvest”.

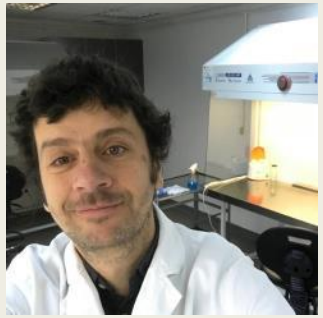
there were activities of:

- preparing medium for growth and multiplication
- working at the laminar flow hood
- noting down and interpreting observations on the plants
- cleaning / disinfecting / sterilizing equipment and instruments

the Material..

- approx. 100 seedlings were used for the experiments - all germinated and grew *in vitro* from *Tanacetum balsamita* L. seeds, harvested out of the spontaneous flora
- the seeds are very small, in length of approx. 2mm, in diameter of approx. 0.3mm - with a shape specific to most seeds of the Compositae / Asteraceae family

..and the Method



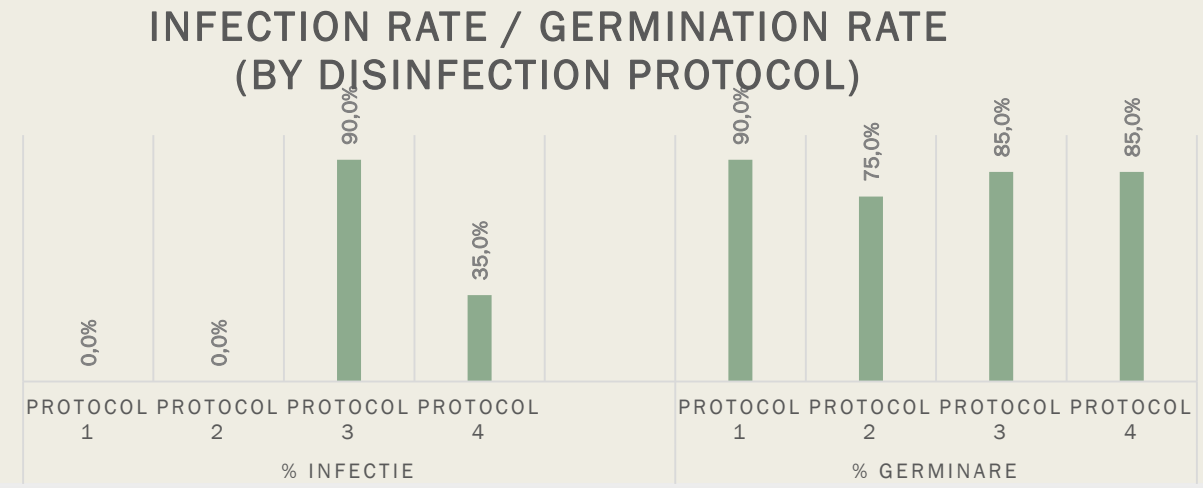
- during the research, experiments and observations were performed on and for:
  - the seed germination capacity
  - the disinfection protocol used before inoculation
  - identifying a favorable growth medium

  and
  - establishing a favorable multiplication medium and multiplication rate

the Results

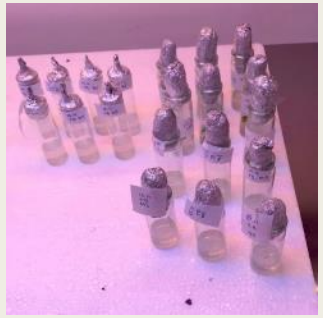
- the GERMINATION test -

- in the *in vivo* **germination test -** no differences were observed in terms of germination capacity in light or dark environment, there were identical rates of 95%.
- the *in vitro* germination test rates: 90% for P1, 75% for P2, 85% for P3 and P4



Results

- the DISINFECTION protocol -

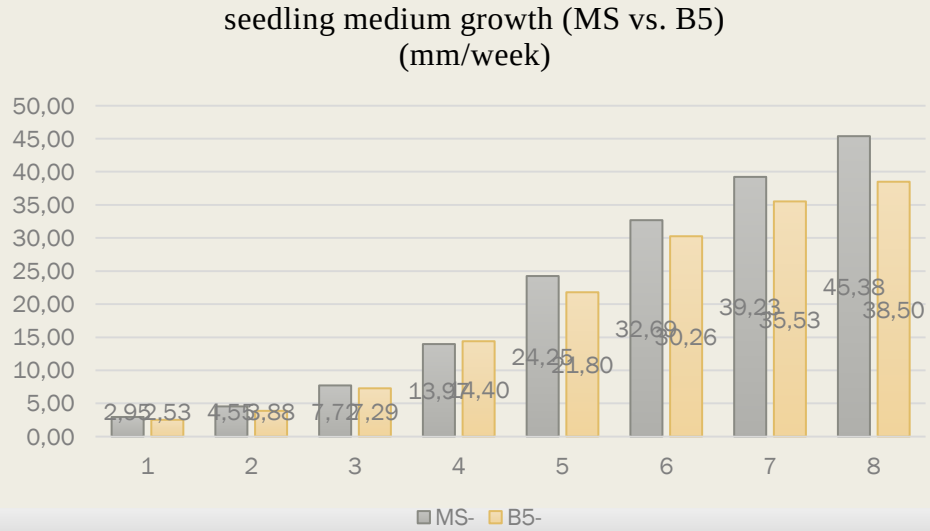


- for **seed disinfection**, the best protocol identified out of 4 proved to be the P1. (Flint fungicide 0.45% - 5 min; rinse 2x in distilled water, sterile - 2x3 min; ethanol 70% - 10 sec; rinse in distilled water, sterile - 3 min; commercial NaClO solution 10% - 8 min; rinse 3x in distilled water, sterile - 1x5 min + 2x3 min.)
- the **total infection rate** after using this protocol (P1) was **0%** and the germination rate for total **germinated seeds** was **90%**

Results

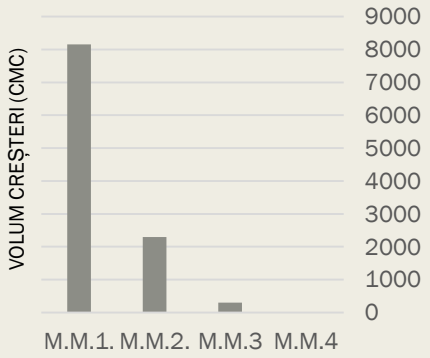
- the CULTURE medium -

- as an **inoculation medium conducive to growth and development**, out of the 2 culture medium chosen to be tested - Murashige et Skoog (MS) without hormones and Gamborg (B5) without hormones - the **MS- medium** proved to be comparatively more **favorable to growth and seedling development** of *Tanacetum balsamita* L



Results

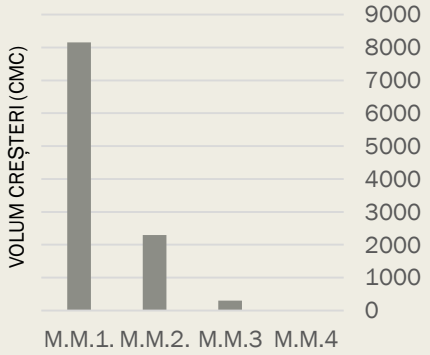
- the MULTIPLICATION medium -



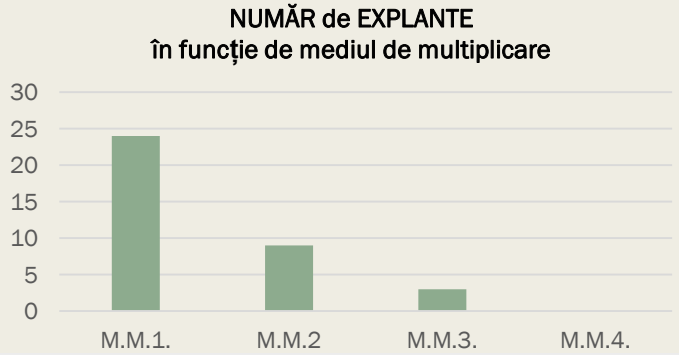
- of the 4 multiplication medium tested, the **best comparative results** were obtained on MM1 (**MS with 1 mg/l IBA; 1 mg/l BAP; 0.5 mg/l GA3**) – there was a total growth volume of 8.150 mm3 and 24 explants were obtained

Results

- the MULTIPLICATION rate -

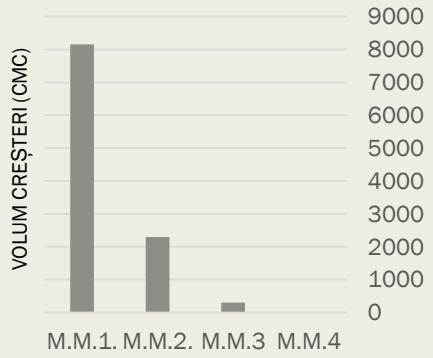


- **the multiplication rate** had also the best results also on MM1 - with a yield of **4.8** explants per transplanted and survived seedling
- **24 explants** were obtained on MM1 vs. **9 explants** on MM2 vs. **3 explants** on MM3 vs. **0 on MM4** – out of 6 seedlings transplanted on each multiplication medium.



Results

- the MULTIPLICATION medium -



- ! **lower hormone concentrations** had much **better effects** on the multiplication and/or growth of transplanted seedlings.

Explant caracteristic -M.M.4

Explant caracteristic - M.M.3

Explant caracteristic - M.M.2.

Explant caracteristic - M.M.1.

Conclusions (and recommendations)

following the experiments on growth, development and *in vitro* multiplication of Tanacetum balsamita L. plants.

- the use for **seed disinfection** of a **protocol** consisting of:
  - 1x Flint fungicide 0.45% - 5 min;
  - 2x distilled water, sterile - 2x3 min;
  - 1x 70% ethanol - 10 sec;
  - 1x distilled water, sterile - 3 min;
  - 1x commercial 10% NaClO solution - 8 min;
  - 3x in distilled water, sterile - 1x5 min + 2x3 min

Conclusions (and recommendations)

following the experiments on growth, development and *in vitro* multiplication of Tanacetum balsamita L. plants.

- for **inoculation, germination, growth** and **development** of seedlings in the first 6-8 weeks - MS culture medium without the addition of any hormones.

Conclusions (and recommendations)

following the experiments on growth, development and *in vitro* multiplication of *Tanacetum balsamita* L. plants

- for the **multiplication of explants** – the use of a MS culture medium with hormones added as follows: 1 mg/l IBA; 1 mg/l BAP; 0.5 mg/l GA3

Thank you!