Protocol – "Blotter test"

- Number of seeds: 400 seeds.

- Proceduress: Seeds should be arranged equidistant on a triple layer of moistened filter paper, inside containers with transparent lids for the light passage, such as petri plates.

- Incubation Conditions: Containers containing the seeds should be placed under white fluorescent lamps in photoperiod for 12 hours, for 7 to 8 days at temperature of 20+/- 2ºC.

- Evaluation: The seeds are examined individually through a stereoscope microscope. Conidiophores with conidia and fruiting bodies (picnids, acerves, perithecia), typical structures of fungi, it was formed in the seeds are important characteristics to identify the fungal species. If necessary, slides should be observed under the optical microscope to confirm the identity of the fungi at the species level. The results should be expressed as percentage of fungi occurrence.

- Comments:

The test is usually used for non-disinfested seeds. If disinfestation is necessary, it should be done with 1% sodium hypochlorite solution for 3 minutes.

The reduction of the germination process of the seeds of dicotyledonous species during the incubation period is done by wetting the paper substrate in 2,4-D solution (2,4-dichlorophenoxyacetate sodium) at 5 ppm concentration. For monocotyledons and some smaller seeds dicotyledons, the freezing technique can be used instead of 2,4-D. In this case, the seeds containers should be incubated for 24 hours at a temperature of 20 +/- 2ºC, then in a freezer (-20ºC) for 24 hours, and finally returned to the incubator at 20+/-2ºC under white fluorescent light for another 5 days.

In rounded seeds case, it is recommended to make small pockets in the paper substrate to prevent them from moving during the handling of the containers. Another option is the incorporation of 0,2% agar to the water used to moisten the paper.

For seeds treated with fungicides the incubation period should be extended for another three days.