Seed sterilization, distribution and conditioning
(10 g L⁻¹ w/v, phosphate buffer 1 mmol L⁻¹, pH 7.5, PPM 0.2%, v/v)

Distribution under stirring of seed suspension
- 50 μL/well; end of 100 μL tips cut

Conditioning
- 7 days, sealed plate, in the dark, 21°C

Seed germination, stimulation and incubation

Dilution plate of tested compounds
- 90 μL/well of water:acetone (99:1; v/v)
- solubilization of tested compounds at 10⁻² mol L⁻¹ in acetone
  - dilution at 10⁻⁴ mol L⁻¹ in water
  - dilution 10x in plate, triplicate, from 10⁻⁵ to 10⁻¹² mol L⁻¹ (from line A to H) in acetone 1%, transfer 10 μL from a line to the next, then homogenize

Stimulation of germination
- add 40 μL/well of water in conditioned seeds plate
- add 10 μL/well of compounds 10x from dilution plate

Incubation
- 4 days, sealed plate, in the dark, 21°C

MTT reduction and control under binocular microscope

- add 10 μL/well of MTT solution
- 24 h incubate, in the dark, not sealed plate, 21°C
- determination of maximum and minimum percentage of germination under a binocular microscope (6 wells)

Solubilization of formazan salts and absorbance reading

- add 200 μL/well of solubilization buffer
- 22h of solubilization, in the dark, sealed plate 30°C, 150rpm
- absorbance reading (570 and 630 nm: A₅₇₀/A₆₃₀)

Extrapolation for germination rate determination

- dose-response analysis and EC₅₀ determination

![Graph showing MTT reduction and control under binocular microscope]

![Graph showing solubilization of formazan salts and absorbance reading]

![Graph showing extrapolation for germination rate determination]