Species:	Microdochium nivale var.nivale or majus
Product Class(es):	SBI and SDHI fungicides
Method type described:	Microtiter plate
Date of protocol:	2022-01
Version	1
comments	This work instruction establishes the procedure for cereal snow mold in-vitro testing of SBI (DMI) and SDHI, but it is suitable for other mode of actions

Sample Collection, Processing and Propagation

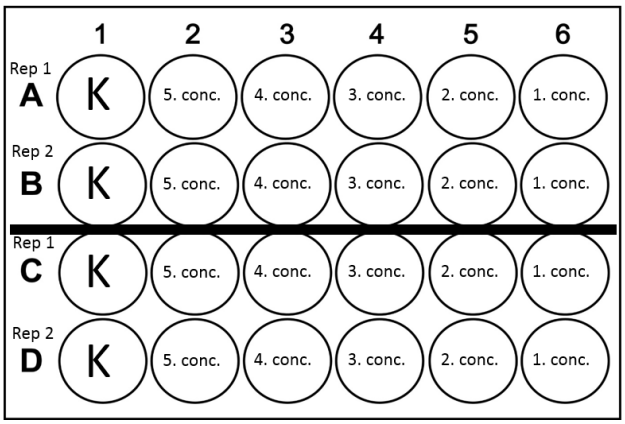
The cereal kernel must wash for 2 minutes with Natriumhypochlorit (3%) to disinfect them. After the disinfection the kernel must wash twice for 2 minutes with bidest. H_2O. Disinfected kernel are taken on a tripartite Petridish with Saboraud maltose Agar (SMA) or Potato Dextrose Agar (PDA) with Streptomycin and Ampicillin and incubate at 20°C under UV light for 7 days. It is important to transfer an agar plug to a new SMA-Plate (mother plate) to get a pure culture for the test.

Sensitivity Tests

Prepare the fungicide dilution series at 10 times higher than the end concentration

Fungicide	**1. conc.**	**2. conc.**	**3. conc.**	**4. conc.**	**5. conc.**	**6. conc.**
Sedaxane	100	10	1	0.1	0.01	0
Prochloraz	100	10	1	0.1	0.01	0

Pipette 100µL of the corresponding fungicide solution in each well of a 24 well plate and add 900µl medium to all wells.



Use a sterile object slide to scrape the fungus from the mother plate and taken into sterile deionised H2O and well mixed. The spore suspension should have a density of 10'000 spore/ mL (count with a Neubauer haemocytometer or a Thoma cell counting chamber). Each well will be inoculated with 50µL spore suspension. The plates are incubated for 6 days at 20°C in the dark.

Evaluation and Data Processing

Evaluation is performed after 6 days at 20°C after inoculation.
Percent growth is assessed visually and EC50 is calculate. EC50 value is estimated for example using a statistic program (e.g. AGSTAT computer package).

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