Species:	*Rhizoctonia solani*
Product Class(es):	QoI and SDHI fungicides
Method type described:	Microtiter plate
Date of protocol:	2022-01
Version	1
comments	This work instruction establishes the procedure for Potato Black Scurf in-vitro testing, but it is suitable for R. solani isolated from other host like sugarbeet and cereals host

Sample Collection, Processing and Propagation

Potato tuber preparation, wash Potato tubers with H2O to remove soil. Cut out scurf's and wash with Natriumhypochlorit (1 minute 1%) to disinfect them. After the disinfection the scurf's are washed twice for 2min with sterile H2O. Disinfected scurf's are taken on a tripartite petri dish with modified Ko and Hora medium and incubate at 20°C in the dark for 7 days. Transfer an agar plug with Rhizoctonia solani to a new PDA-Plate and incubate for 7-10 days at 20°C in the dark to get a pure culture.

<u>PDA for 1000ml</u>

- 39g Potato dextrose Agar.

Weigh PDA and adjust with purified H_2O to 1000ml. Autoclaved 20 min at 121°C. Cool down to 50°C.

<u>Modified Ko and Hora for 1000ml</u>

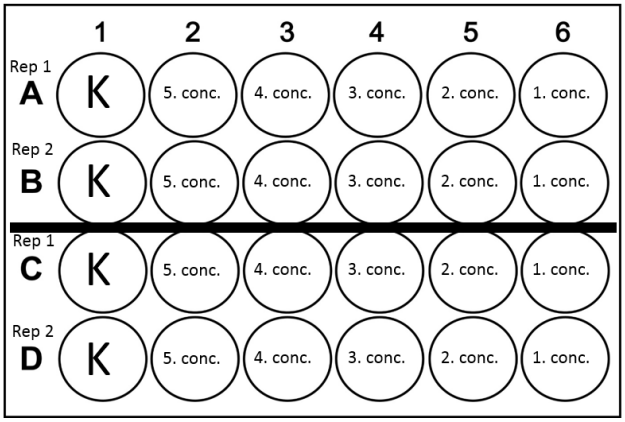
- 1g K_2HPO_4
- 0.5g $MgSo_4\ 7H_2O$
- 0.5g KCl
- 0.2g $NaNO_2$
- 50mg Chloramphenicol (dissolve 50mg in 0.5ml EtOH 100%)
- 50mg Streptomycin (dissolve 50mg in 1ml sterile H2O)
- 5mg Mefenoxam (0.5ml of 10'000ppm stock solution)
- 5mg Prochloraz (0.5ml of 10'000ppm stock solution)
- 20g Bacto Agar

Weight salts + agar and solute them in ~800ml purified H_2O. Adjust with purified H_2O to 1000ml. Autoclaved 20ml at 121°C. Cool down to 50°C.
Add 50mg Chloramphenicol, 50mg Streptomycin, 0.5ml Mefenoxam and 0.5ml Prochloraz (10'000ppm stock solution) and mix gently

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	10	1	0.1	0.01	0.001	0
Azoxystrobin	10	1	0.1	0.01	0.001	0

Pipette 100µl of each concentration (quadruplicate) in 24 well plate
Add 900µl of PDA to each well and mix gently



Punch out 1mm rondels from strain pure culture plate and place into center of each of the 24 wells of the plate. Incubate Plates for 4 days at 20°C in the dark.

Evaluation and Data Processing
Evaluation is performed after 4 days at 20°C.
Percent growth is assessed visually and the EC50 value estimated for example using a statistic program (e.g. AGSTAT computer package).

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