Species:	*Corynespora cassiicola*
Product Class(es):	QoI fungicides
Method type described:	Microtiter plate
Date of protocol:	2026-06
Proven for	Metyltetraprole
Version	1
comments	Protocol adjustments may be needed for other fungicide classes depending on the individual compound characteristics.

Method:

1. Sampling: To obtain *Corynespora cassiicola* isolates, soybean or cotton leaves showing visible disease symptoms are collected. Young leaves with newly developed lesions are preferable. The leaves should be dried thoroughly to remove any surface moisture, placed individually between dry paper towels, and sent to the laboratory.

2. Inoculum production: Conidia from these soybean or cotton leaves are transferred onto the surface of Petri dishes containing potato dextrose agar (PDA). The plates are incubated at 23 ºC in the dark. A small piece of a mycelium is then transferred to V8 agar medium and incubated at 23 ºC in the dark until sporulating colonies of *C. cassiicola* develop. Conidia are harvested by flooding the plates with sterile distilled water and gently rubbing the surface with a sterile glass rod. The suspension is filtered through sterile gauze to remove mycelial fragments and then adjusted to $1x10^3$ conidia / mL in YBA medium* containing 400 µM n-propyl gallate.

3. Sensitivity tests: The technical-grade active ingredient is dissolved in dimethyl sulfoxide (DMSO). A 100-fold dilution series of the active ingredient at the following final concentrations is prepared: 0, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 ppm. Aliquots (1 µL) of each dilution are mixed with the inoculum (99 µL) in 96-well microtiter plates. The microtiter plates are covered with plastic wrap to prevent evaporation and incubated at 23 ºC in the dark.

4. Evaluation: Two to three days after incubation, fungal growth is assessed by measuring the optical density at 600 nm using a microplate reader. The values are corrected using the blank well without inoculum. The 50% effective concentration (EC_{50}) is calculated by logit regression analysis.

*Yeast Bacto Acetate medium
10 g Yeast extract
10 g Bacto peptone
20 g Sodium acetate
fill up to 1000 mL with distilled water

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