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Protocol status: Working
We use this protocol and it's working

Baiting Pythium myriotylum from Infested Soil V.2

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ABSTRACT

Baiting *Pythium* from seedlings in infested soil or infected hulls.

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MATERIALS

P_5ARP plates
CMA/PDA+amp plates
Soil with *Pythium* infestation signs
Seeds of susceptible peanut cultivar; we used PI 378012 from the USDA Germplasm Collection
Fume hood and tools for sterile plating technique
Sieve and beakers for surface sterilization
RO water
Forceps and scalpel/blade
95% ethanol
Flame

For seedling baiting:
4" pots with catch tray
greenhouse or adequate outdoor conditions
Mature banker plants (Alyssum and Peppers) for IPM

Preparation

1 Prep P_5ARP Plates 1-2 days before plating.

Prepare working culture plates (CMA, PDA + amp) up to 1 week before plating.

Seed Pod or Seedling Baiting

8m

2 Select whether you want to bait from diseased hulls in soil or from seedlings grown in diseased soil.

STEP CASE

Peanut Seedlings 14 steps

To bait from soil infested with Pythium using susceptible germinated seedlings.

3 Germinate susceptible seedlings by wrapping in RO-dampened paper towels and incubating for 2-4 days at 28 °C in the dark.
Check daily to re-wet paper towels as necessary.

4 Plant germinated seeds in infested soil, in triplicate, in 4" diameter pots with a drip tray. Maintain greenhouse at 70 % humidity with temperatures between 24 °C and 30 °C.

4.1 Water to maintain a level of 1-2" in drip tray for the first 2 days, and then to maintain soil moisture without excessive water until seedlings reach 4-6" in height.

5 At 4 DAP, trim to 1 seed/pot, or carefully repot in separate pots.

6 Grow for 15-20 days and check for rot symptoms. Brown rot will start at ~4-6 DAP, wilt at ~6-18 DAP. Those exposed to higher temperatures may be more susceptible to rot.

Harvest and Surface-Sterilization

7 Harvest seedlings. Rinse plants thoroughly in RO water to remove soil particles.

8 Select appropriate samples with black rotted segments.

Rotted segments of peanut seedling suitable for plating

8.1 Cut up segments into 1/4 inch (1-2 cm) pieces.

9 Try with or without [M] 95 % (v/v) ethanol soak for 00:04:00 or rinse in sterile RO water. Use a sieve 4m
in a small beaker.
Do not use bleach due to *Pythium* sensitivity. [2]

9.1 Blot dry on sterile paper towels.

10 Plate on P_5ARP or other media with bacterial inhibition (CMA or PDA + amp) [4].

Transfer Culture

11 Check plates after 24-48 h.

12 Hyphal tip transfer using sterile pin to clean plates of CMA (V8 or PDA).

Oospore Check

13 Check for "gold coin" oospores at plate edges to indicate *Pythium myriotylum*.

- Use CMA for oospore production
- Use full strength PDA to increase hyphal growth for DNA extraction
- Use V8 to increase hyphal growth for DNA extraction (untested)