

# **Combined Antimicrobial Protocol for Fungi & Nematodes Using 100 nm AgNPs**

## **Objective**

To evaluate and apply silver nanoparticles (~100 nm) as a dual-action treatment against plant fungal pathogens (e.g., *Fusarium*, *Rhizoctonia*) and soil nematodes (e.g., *Meloidogyne*, *Pratylenchus*) using a standardized, scalable method.

## **Materials**

- 100 nm AgNP stock solution (known concentration, ideally ~770 ppm)
- Deionized water
- Optional additives:
  - 0.01% Tween 20 (wetting agent for foliar adhesion)
  - 5% glycerol (to enhance cuticle penetration)
  - Bio-extract or capping agent (e.g., plant extract, PEG, or spermidine)
- Graduated cylinder or micropipette
- Spray bottle or foliar applicator
- Soil drench container or irrigation injector
- Target plant(s), inoculated or infested with fungal or nematode pathogens

## **Formulations**

### **Foliar Spray (Fungi + Airborne Spores)**

AgNP stock (770 ppm): 650 µL

Glycerol (optional): 1 mL (1%)

Tween 20: 100 µL (0.01%)

Water: up to 99.5 mL

Final [AgNP]: ~5.0 ppm

### **Soil Drench (Nematodes + Root-zone Fungi)**

AgNP stock (770 ppm): 2 mL per 100 mL drench

Water: up to 100 mL

Final [AgNP]: ~15 ppm

## **Application Protocol**

### **Prepare Plants**

Choose healthy and infected plants. For nematode trials: pre-inoculate soil with *Meloidogyne* spp. For fungal trials: inoculate leaves with *Fusarium* spores.

## **Foliar Spray**

Spray plants evenly, focusing on leaf surfaces and undersides. Apply until slight runoff; avoid oversaturation.

Best timing: early morning or late afternoon. Repeat every 5-7 days or after heavy rain.

## **Soil Drench**

Apply 50-100 mL of prepared AgNP solution per plant base or per pot. Ensure even wetting around root zone. Repeat weekly for 3 weeks.

## **Optional Evaluation Steps**

**MIC/MFC in vitro - XTT, TTC, or plate assay: Confirm antimicrobial activity**

**Root gall index - Dig and rate roots (1-5 scale): Nematode impact**

**Leaf lesion score - Count lesions, % coverage: Fungal suppression**

**SERS confirmation - Raman scan of leaf surface post-spray: Verify AgNP deposition**

## **Safety and Handling**

Store AgNPs away from light and extreme pH. Wear gloves and mask while spraying. Avoid direct runoff into water bodies.