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## RESEARCH NOTE

# A modified fungicide based media for the isolation of *Phytophthora cinnomomi* Rands. causing avocado root rot

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**ABSTRACT:** The commonly chosen medium for isolating Phytophthora is the P10VP medium, which contains pimaricin (10 mg), vancomycin (220 mg), and PCNB (pentachloronitrobenzene) (125 mg a.i.). Pimaricin has been a longstanding ingredient in the P10VP medium for *Phytophthora* isolation. In this research, we designed a modified isolation medium with propiconazole as the primary component for isolating *Phytophthora* from infected plant samples. This modified medium was tested and validated for various Phytophthora species affecting different crops.

**Keywords**: *Phytophthora cinnamomi*, Pimaricin, Propiconazole

*Phytophthora cinnamomi* Rands, an Oomycete, is known for causing severe root rot and dieback in various agricultural and forestry host plants, including avocado, chestnut, macadamia, oak, peach, and pineapple (Hardham and Blackman 2018; Madhu et al., 2023). The infection primarily targets feeder roots, leading to their decay. Isolating this pathogen from feeder roots is a challenging task due to the presence of numerous saprophytes in decayed feeder roots. Standard media are unsuitable for *P. cinnamomi* isolation as they quickly lead to contamination by other fungi. Therefore, the use of selective media containing P10VP (pimiricin 10 mg, vancomycin 220 mg, PCNB (pentachloronitrobenzene) 125 mg a.i.) or PARP-V8 (20 g agar, 200 ml filtered V8 broth, 800 mL deionized water, 5 mg pimiricin, 10 mg rifampicin, 250 mg ampicillin, and 125 mg a.i. PCNB) has proven effective for the selective isolation of Phytophthora (Tsao and Ocana, 1969).

However, the drawback of these media lies in the use of pimaricin, which is highly photosensitive (Gutteridge et al., 1953). Inoculated plates must be kept dark to prevent photo inactivation-related potency loss. Tsao and Ocana (1969) noted that prolonged storage of the medium in the dark and at low temperatures results in the loss of antibiotic action. Additionally, distinct strains of *Phytophthora* exhibit sensitivity to pimaricin. While pimaricin does not hinder mycelial growth, it strongly or completely inhibits the germination of chlamydospores, sporangia, and zoospores in many tested species (Jeffers and Martin 1986). Against this background, the present study aims to develop a fungicide-based medium as an alternative to the antibiotic pimaricin for isolating *Phytophthora* from infected plant samples. Attempts were made to isolate *Phytophthora* infecting avocados using Corn Meal Agar (CMA) supplemented with specific active ingredients. The composition of CMA per litre included Pr1VRP (propiconazole 25% EC 1ml, vancomycin chloride 200mg, rifampicin 10mg, PCNB 100mg) and Pr2VRP (propiconazole 25% EC 2mL, vancomycin chloride 200mg, rifampicin 10mg, PCNB 100mg), which were added after autoclaving. Infected avocado feeder roots displaying symptoms of dark, bristly roots were selected for organism isolation. The collected feeder roots were cleaned in sterile distilled water, air-dried, and then inoculated for isolation. Surface sterilization using alcohol or other chemicals was avoided to prevent occasional inhibition of *Phytophthora* growth. After seven days of incubation at 22°C, plates were examined for mycelia development and chlamydospore production.

*Phytophthora cinnamomi* produces coralloid hyphae with grape-like clusters of chlamydospores that grow laterally on the hyphae (Hardham and Blackman 2018). Colonies displaying cottony growth at the tip of growing mycelium were selected and subcultured to obtain a pure culture of the organism (Fig 1). The CMA medium containing propiconazole did not inhibit the mycelial growth and chlamydospore formation of *Phytophthora* in the culture medium. However, at a propiconazole concentration of 2 ml/L, the growth of saprophytic

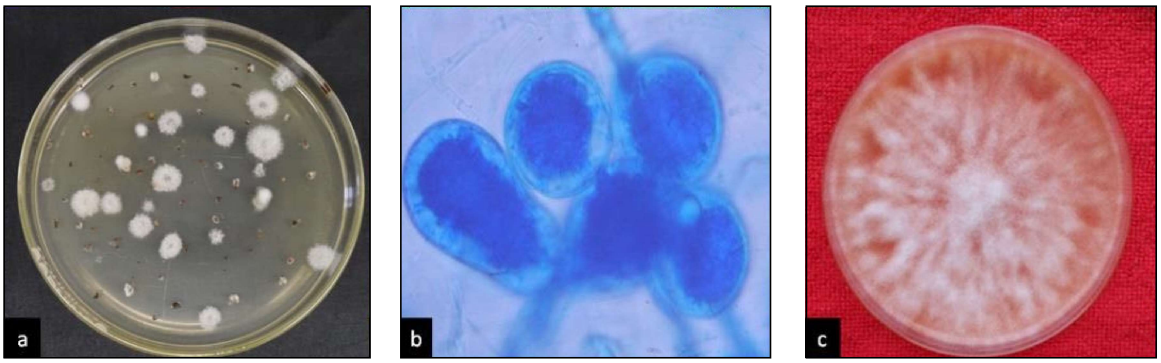


**Fig 1. *Phytophthora cinnomomi* a. Whitish mycelial growth of *P. cinnomomi* after avocado root inoculated on Pr2VRP medium b. Chlamydospores on Pr2VRP medium c. Ten days old culture on PDA media**

fungi was completely inhibited, successfully recovering Phytophthora. The antibacterial chemicals rifampicin + vancomycin chloride proved more effective than rifampicin alone at inhibiting bacterial development in the culture medium. After one week of incubation at 25°C, no bacterial colonies were observed on any inoculated plates with Pr1VRP or Pr2VRP. Pr2VRP emerged as the most effective chemical for isolating *P. cinnomomi* from infected feeder root samples. This medium, however, has not been tested for the isolation of *Phytophthora* from soil samples. Pr2VRP medium has also been standardized to isolate Phytophthora infecting black pepper roots, cacao pods, guava fruits, and papaya roots. Propiconazole, a triazole-based fungicide used to combat true fungal diseases, acts by inhibiting ergosterol biosynthesis, which is crucial for fungal cell wall formation. However, Propiconazole does not inhibit the growth of Oomycetes such as *Phytophthora* and *Pythium*, as their cell walls contain cellulose, and ergosterol is not a major sterol in the cell membrane. Fungicides targeting chitin and ergosterol synthesis are generally ineffective against Oomycetes (Gad *et al.,* 2014). Using Propiconazole for Phytophthora isolation is cost-effective, readily available in the market, and can serve as an alternative to pimaricin.

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