A simple baiting technique to detect and isolate *Phytophthora capsici* (*'P. palmivora'* MF_4) from soil

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A rapid technique for detection and isolation of *Phytophthora capsici* (*'P. palmivora'* MF_4) from soil is described. It facilitates implementation of early control measures.

Key words: *Phytophthora capsici*, Baiting, Techniques.

Phytophthora diseases on black pepper (*Piper nigrum* L.) in India occur mainly during the south-west monsoon period (June–Sept.) when weather conditions are favourable (Anandaraj, Ramachandran & Sarma, 1988). During the inter-monsoon dry period the fungus remains inactive in the soil. The exact mode of survival is not clearly understood, although survival in the form of chlamydospores, inactive mycelium, oospores, etc., have been postulated (Sarma & Nambiar, 1982; Sastry & Hegde, 1988). Detection and isolation of this fungus from soil poses serious problems. There are several baiting techniques and selective media to isolate and quantify *Phytophthora* from soil (Tsao, 1983; Dingra & Sinclair, 1985).

To isolate *Phytophthora* spp. responsible for diseases of black pepper from soil, baits such as apples (Holliday & Mowat, 1963), black-pepper leaves (Kueh & Khew, 1982), black-pepper leaf discs (Ramachandran *et al.*, 1986) and castor seeds (Sastry & Hegde, 1988) have been used. In these cases positive baiting could be confirmed only after subsequent plating of the infected baits on selective media. In the present study *Albizia falcataria* (L.) Fosberg leaflets have been used as baits to isolate black pepper *Phytophthora* from soil. The advantage of this method is that the fungus infects the baits and sporulates profusely on the bait within 72 h, which enables easy confirmation of positive baiting.

The test soil was sieved to < 2 mm fractions, 25 g placed in a Petri dish and 40–50 ml of glass distilled water added to make a soil–water suspension, so that 2–3 mm of free water stood above the soil when the particles settled. Two replicates of twenty leaflets of *Albizia falcataria* were floated with the adaxial surface in contact with water. Plates were incubated in the laboratory at 26° (±2 °C) for 72 h. Depending on the density of the inoculum, all or some of the baits became infected. The infected baits turned black and mycelium emerged at the edges and sporulated. If all the baits were not infected within 72 h, they all became infected subsequently, due to release of zoospores. To isolate the fungus into pure culture, infected baits were surface-sterilized with mercuric chloride 0·1% for 1 min, rinsed with four changes of sterile water and plated either on to water agar or PVPH selective medium (Tsao & Guy, 1977). The infected sporulating baits, when placed on black-pepper leaves and incubated in a humid chamber, produced characteristic lesions with fimbriate margins within 48 h. Tsao (1983) suggested the use of a large quantity of water in a beaker instead of Petri dishes. His results were confirmed in the present studies.

Test soil, 300 g, was taken and half of this was distributed into six containers of 25 g each (two Petri dishes and four polythene containers). The remaining 150 g soil was used for making a serial dilution with autoclaved soil to give a final dilution of 1/2048 – the serial dilution end-point method (Tsao, 1960). Soil, 25 g, from each dilution was placed in one of the six containers. Different quantities of water ranging from 25 to 400 ml were added and baited with *Albizia falcataria* leaflets. The Disease Potential Indexes (DPI), defined as the reciprocal of the highest dilution which produce positive baiting under test conditions (Tsao, 1960) obtained, are given in Table 1.

Soil:water (ratio 1:4) gave the highest DPI value and this ratio is being used for further studies. The baiting efficiency of *Albizia falcataria* leaflets was compared with three other baits, namely *Leucaena leucocephala*, young and mature leaf discs of black pepper. The DPI values obtained are given in Table 2. The advantages which *A. falcataria* leaflets as baits have over others are: greater sensitivity compared with pepper-leaf discs, and direct sporulation on the baits, facilitating easy

Table 1. Disease potential index obtained with *Albizia falcataria* leaflets with different quantities of water

Treatment	Quantity of soil / Quantity of water	Disease potential index: Sample 1	Sample 2
A	25 g + 25 ml water	128	2
B	25 g + 25 ml water	128	1
C	25 g + 50 ml water	256	1
D	25 g + 100 ml water	512	4
E	25 g + 200 ml water	64	1
F	25 g + 400 ml water	128	1

Table 2. Disease potential index of a contaminated field soil with different baits

Bait	Disease potential index
Albizia falcataria leaflets	8
Leucaena leucocephala leaflets	2
Black pepper: young leaf discs	1
Black pepper: mature leaf discs	1

infection confirmation, which reduces time. Since whole leaflets are used, saprophytic colonization is minimized. However, after the tissue is dead it becomes colonized by *Pythium* and other saprotrophs and should not be used longer than 5 d. The presence of typical sporangia confirms positive baiting by *Phytophthora*. In black pepper, if the root system is infected, it takes several weeks before the aerial symptoms become visible, when it may be too late for any control measures to be taken. By this method, once the presence of pathogen and the DPI has been established, control measures could be taken earlier, preventing infection.

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Pseudocercospora gymnosporiae sp. nov. from India

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Pseudocercospora gymnosporiae sp. nov. from India. *Mycological Research* **94** (7): 1004–1005 (1990).

Pseudocercospora gymnosporiae sp. nov. is described and illustrated from leaves of *Gymnosporia spinosa* and compared with published accounts of similar species.

Key words: *Pseudocercospora gymnosporiae*, *Gymnosporia*, New species.

Pseudocercospora gymnosporiae R. K. Dubey *et al.* (Fig. 1)

Maculae amphigenae, circulares fere irregulares vel interdum ad maculae coalescentes, coronas brick rubido. *Colonae* amphigenae, limitatae maculae, distinctae punctiformes fusco glauco brunneae. *Mycelium* ex hyphis immersis ramosis septatis compositum. *Stromata* bene evoluta partim immersa et partim erumpentia, interdum superficialia, pseudoparenchymatica, medio usque fusco olivacea, 22–148 µm. *Conidiophora* caespitosa, macronematosa, mononematosa erecta vel suberecta, septata, eramosa, glabra, subflexuosa, pallide olivacea usque medio olivacea, 20–185 × 3·5–6 µm. *Cellulae*

Table 1. Comparison between *Pseudocercospora ochnae* and *P. gymnosporiae*

	Stroma	Conidiophores		Conidia		
		Structure	Colour and size	Structure	Colour, septation	Size
P. ochnae	Well developed, 20–100 µm wide	Loose fascicles in groups, erect, often flexuous, unbranched, geniculate with conidial denticles, denticles short and basal scars not thickened	Subtly dune to pale brown, 100–250 × 4·5–8·5 µm	Obclavate	Brown to olive brown, 2–9	40–150 × 4–10 µm
P. gymnosporiae	Well developed, 22–148 µm wide and to dark olivaceous	Fasciculate, erect to sub-erect, unbranched, not denticulate	Light to mid olivaceous, 20–185 × 3·5–6 µm	Obclavate cylindric to cylindric or lenticular	Light olivaceous, Up to 28	9–180 × 2·5–6 µm