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A new and simple baiting technique for easy isolation of *Phytophthora palmivora* Butl. from bud rot affected tissue of coconut

K.M. Sharadraj and R. Chandra Mohanan*

*Central Plantation Crops Research Insitute (ICAR), Kasaragod 671124 Kerala, India. *E-mail: rcmcpcri@yahoo.co.in*

Abstract

Earlier studies using different methods of isolations revealed that it is extremely difficult rather impossible in certain cases to isolate the pathogen, *Phytophthora* sp. from bud rot disease of coconut due to the presence of high population of other microorganisms like bacteria and fungi in the rotten tissue. Hence, it was very much imperative to develop an easy technique for isolations of *Phytophthora* sp. from samples collected from various locations. In the modified baiting method, different treatments were given to infected tissues before baiting with susceptible healthy plant tissues. Among the different treatments given to infected tissue, the treatment of keeping infected tissue in carbendazim (Bavistin 50 WP) 125 ppm + rifampicin 200 ppm solution was found to be better for successful infection of baits and successive isolation of *Phytophthora palmivora* (Mean 30%) irrespective of the baits used. Out of the seven types of baits used in this treatments, rachillae of young unopened coconut inflorescence, leaves of *Loranthus parasiticus* (L.) Merr. and leaves and young fruits of badam tree (*Terminalia catappa* L.) were found to be superior to others (with 33-37% success in isolation) and they were statistically on par. Tender leaves of badam tree yielded the highest percentage (37) of isolation of *P. palmivora*, when used as bait in the treatment carbedazim + rifampicin solution containing rotten bud tissue of coconut palms. Hence it can be used for large scale isolation of *P. palmivora.*

Key words: Baiting, coconut, bud rot disease, *Phytophthora palmivora.*

Introduction

Bud rot and premature nutfall caused by *Phytophthora palmivora* are major disease problems affecting coconut palms (*Cocos nucifera* L.) in India and account for extensive losses of both stand and nut production. Of these, bud rot is a fatal disease occurring in all coconut growing countries, owing to the great economic loss it causes year after year particularly in years with heavy and continuous rainfall. The disease intensity varies from locality to locality as well as from garden to garden depending on the climatic conditions and source of inoculum (Sharadraj and Chandra Mohanan, 2013).

Isolation of the causal organism from disease affected bud rot tissue is very difficult and often not possible by direct isolation method due to the interference of other microorganisms in the rotten tissue (Drenth and Guest, 2004; Uchida, 2004). As soon as *Phytophthora* infection starts in the bud tissue, other microorganisms such as fungi and bacteria enter the tissue as secondary invaders posing problems in *Phytophthora* isolation. Since most of the *Phytophthora* species are difficult to isolate from decayed tissue or from soil, baiting was the isolation method initially developed for isolation of *Phytophthora* from diseased tissue. But baiting methods used were also not very successful in the case of bud rot of coconut, especially when the tissue is very much rotten or in advanced stages of the disease (Rasmi, 2003). Considering such problems, some selective culture media containing chemicals to inhibit the growth of other microorganisms present in the infected tissue were developed to isolate *Phytophthora* sp. (Jeffers and Martin, 1986). Drenth and Sendall (2004) reported that hymexazol in selective medium inhibited most of the species of *Pythium.* But it also inhibited some *Phytophthora* sp. Hence, they suggested to use selective media with and without hymexazol. The method of isolation of *Phytophthora* species from coconut bud rot tissue has been reported by only some workers and therefore the information on this is very scanty.

The constraints reported from earlier studies in isolating *Phytophthora* from bud rot affected tissues point to the need to undertake systematic studies to develop an easy and simple technique for *Phytophthora* isolation.

Materials and methods

In order to standardise the method of isolation of *P. palmivora* from bud rot disease affected tissue of coconut, the fully rotten tissue which formed a slimy mass was completely removed from the samples collected and other infected portions of the bud tissue were used on the same day of collection. The infected tissues were cut into small pieces using a sterilized knife, kept in 140 mm size Petri plates and following treatments were given before baiting.

Treatments

1. Carbendazim (Bavistin WP) 125 ppm solution was poured over the infected tissue pieces in Petri plates so as to just cover upper surface of the pieces.
2. Infected tissue pieces in Petri plates were treated with Carbendazim (Bavistin WP) 125 ppm and rifampicin 200 ppm solution as in treatment 1.
3. Sandy loam soil of a bud rot disease free coconut (West Coast Tall) garden of about 20 years old was collected from the upper layer (up to 10 cm from ground level) removed pebbles, roots and other

particles if any, moistened with water, sterilized at 121ºC for 1 h. and allowed to cool to room temperature.The infected tissue sample was mixed with moistened and sterilized soil in Petri plates.

4. Infected tissue pieces were kept in sterile water as in the case of fungicide solution.

To find out effect of the treatments in causing infection on baits, healthy coconut tissues such as buttons (one-month-old nuts), pieces of tender leaflets from unopened spindle leaf, inflorescence rachillae and male flowers from young unopened inflorescence, and also leaves of plant parasite, *Loranthus parasiticus* L. and pieces of healthy tender leaves and young fruits of *Terminalia catappa* L. (Indian almond/ Bengal badam/badam tree) (Fig. 1 a and b) were used as baits in each of the four treatments. The badam trees, found in the vicinity of coconut gardens were showing severe incidences of *Phytophthora* leaf blight and fruit rot leading to defoliation and immature fruitfall during south-west monsoon period.

Loranthus was found as a common parasite in many trees especially mango trees in the southern part of India. *Phytophthora* infection of leaves of *Loranthus* leading to severe defoliation was observed very commonly in the high rainfall areas of South India. Hence, the healthy tissues of both these plants were also included as baits to isolate *Phytophthora* spp. from bud rot affected tissue of coconut. All the baits without any symptoms of infection or insect attack were collected without making any injuries or bruises on the surface, brought to laboratory in sterile polythene bags and used on the same day of collecetion. Healthy coconut samples were collected from 20-25 years old West Coast Tall palms, a variety commonly cultivated in Kerala State. Thus, seven healthy plant parts were used as baits in each of the four treatments containing infected tissues. The baits were thoroughly washed in water and wounded with pin pricks using sterilized entomological pins before keeping in the 4 treatments. Three replications of each treatment were maintained for each bait with 10 units of each bait per replication. The baited Petri plates were kept inside a plastic tray of 45.50 x 33.00 cm size containing wet cotton balls to provide high humidity (100% RH), closed the tray with a polythene sheet and incubated at 26 ± 2ºC. The baits were observed every day and whenever lesions of infection were noticed on the surface of the baited tissue it was used for isolation of the causal organism. For this, the infected baits were washed and cleaned with water. Pieces (about 6 x 5 mm size) of infected tissues from the advancing margin of the lesion were cut with a sterile razor blade, surface sterilized with 0.1% mercuric chloride solution followed by washing in three changes of sterile water and plated on carrot agar medium. The percentage of baits in each treatment yielding *Phytophthora* spp. was found out by isolation and identification of the causal organism from the lesion developed on the baits.

Results and discussion

Among the four treatments given to bud rot affected tissue (*P. palmivora*) before baiting, the treatment with carbendazim (Bavistin 50 WP) 125 ppm + rifampicin 200 ppm solution was found to be better than all other treatments in getting successful isolation of *Phytophthora palmivora* from the baits irrespective of the baits used. Among the seven types of baits used for isolation of *P. palmivora* from the lesions developed on the baits used in carbendazim + rifampicin treated infected tissue, rachillae of young coconut inflorescence, *Loranthus* leaf and tender leaf pieces and immature fruits of badam tree were superior to other baits in yielding *Phytophthora* and they were statistically on par with 33 to 37 % success of isolation (Fig. 2). Of these, badam leaves yielded the highest percentage (36.66%) of successful isolation. When one to two months old immature coconuts were used as baits, 23.33% of the nuts yielded *Phytophthora.* Tender leaf from unopened spindle and male flowers of coconut were not found to be good baits as only 10 and 16.66% of these respectively yielded *Phytophthora* spp. (Table 1). Concibido-Manohar (2004) reported that, though *Phytophthora* was found to be the primary pathogen, its place in the tissue was soon occupied by bacteria like *Erwinia*, which caused maceration of the tissue. This was often pointed out as the reason for the difficulty in isolating the pathogen in cases of advanced rot.

Table 1. Standardization of isolation of *Phytophthora* spp. of coconut bud rot disease by baiting method

Sl. No.	Treatment	Percentage of baits yielded *Phytophthora* spp.							
		Coconut				Loranthus leaf tissue	Badam tree		Mean
		One to two-months-old nuts	Tender leaf disc/pieces	*Inflorescence rachillae pieces	Male flower		Tender leaf bits	Immature green fruits	
1	Infected tissue + Carbendazim solution (Bavistin 125 ppm)	16.66 (23.37)	10.00 (18.44)	10.00 (18.44)	10.00 (15.01)	13.33 (21.16)	16.66 (23.87)	20.00 (26.58)	13.81 (20.98)
2	Infected tissue + Carbendazim solution (Bavistin 125 ppm) + rifampicin 200 ppm	23.33 (28.79)	10.00 (15.01)	33.33 (34.94)	16.66 (23.37)	33.33 (34.65)	36.66 (37.16)	30.00 (33.02)	26.19 (29.56)
3	Infected tissue + moistened and sterilized garden soil	10.00 (18.44)	0.00 (0.00)	6.67 (12.30)	3.33 (6.15)	20.00 (25.38)	23.33 (28.79)	20.00 (25.38)	11.90 (16.63)
4	Infected tissue + SDW	3.33 (6.15)	0.00 (0.00)	6.66 (12.30)	0.00 (0.00)	0.00 (0.00)	6.66 (12.30)	3.33 (6.15)	2.85 (5.27)
	Mean	13.33 (19.19)	5.00 (8.36)	14.16 (19.49)	7.50 (11.13)	16.66 (20.30)	20.83 (25.53)	18.33 (22.78)	
For treatments LSD (P=0.05)		5.12							
For baits LSD (P=0.05)		6.77							

* From young and unopened inflorescence

SDW : Sterile distilled water.

Treatment 1 to 3 : Infected tissue cut into small pieces and mixed with SDW or other solutions in sterile Petri plates so as to partially cover the upper surface of the tissue.

Figures in parenthesis are angular transformed values

Fig. 1. A: Healthy leaves of *Loranthus* B: Leaves and fruits of *T. catappa*

Though, Rasmi (2003) could isolate *P. palmivora* from rotten bud tissue of coconut by baiting unopened male flowers of coconut inflorescence, high frequency of isolation was obtained only when freshly infected tissues that had not been affected by secondary invaders were used. However, it is very difficult, rather impossible to recognize such tissues visually. The present study clearly revealed that male flower of coconut inflorescence was not good bait compared to other baits used in isolating *Phytophthora* spp. from bud rot affected samples.

Among all the treatments, infected tissue in sterile distilled water was the least effective treatment in causing infection on baits and in yielding *Phytophthora* irrespective of the baits used. In this treatment also, inflorescence rachillae and tender badam leaves (baits) gave the highest percentage (6.66%) of successful isolation of *Phytophthora* compared to other baits used. The bud rot samples used for standardization of isolation method was taken from a single coconut palm and hence all isolations yielded only *P. palmivora.*

Thus, it has been conclusively proved that badam leaves and immature fruits are good baits for easy isolation of *P. palmivora* from bud rot affected tissue treated with carbedazim and rifampicin.

Drenth and Guest (2004) also reported that it was not possible to isolate *Phytophthora* from palms with advanced bud rot due to bacterial decay of the *Phytophthora* infected bud. Therefore, they recommended using tissue from the advancing margin of the lesions in the very initial stage of the disease; as such tissues were relatively free of secondary invaders. Though, several workers recommended and used selective media for *Phytophthora* isolation (Tsao and Ocana, 1969; Masago *et al*., 1977; Ribeiro, 1978; Papa*v*izas *et al.,* 1981; Jeffers and Martin, 1986; Uchida *et al.,* 1992), there are several constraints like high cost of chemicals, non-availability of some of the chemicals at the place of study. The techniques is also useful, when large number of isolations are to be undertaken for collection of the pathogen from various locations for studies like pathogen diversity, selection of virulent strains *etc*. On the other hand, Drenth and Sendall (2004) considered baiting as a very relevant technique in *Phytophthora* isolation because the initial steps in isolation of the pathogen can be performed in the orchard and surface sterilization of the baits is usually not very essential.

Thus, baiting technique developed was found to be very ideal and easy method for isolation of *Phytophthora* spp. from bud rot disease affected tissue contaminated with secondary invaders and it was much cheaper than isolation using selective media. It is also very much essential to treat the infected tissue prior to baiting to suppress other microorganisms.

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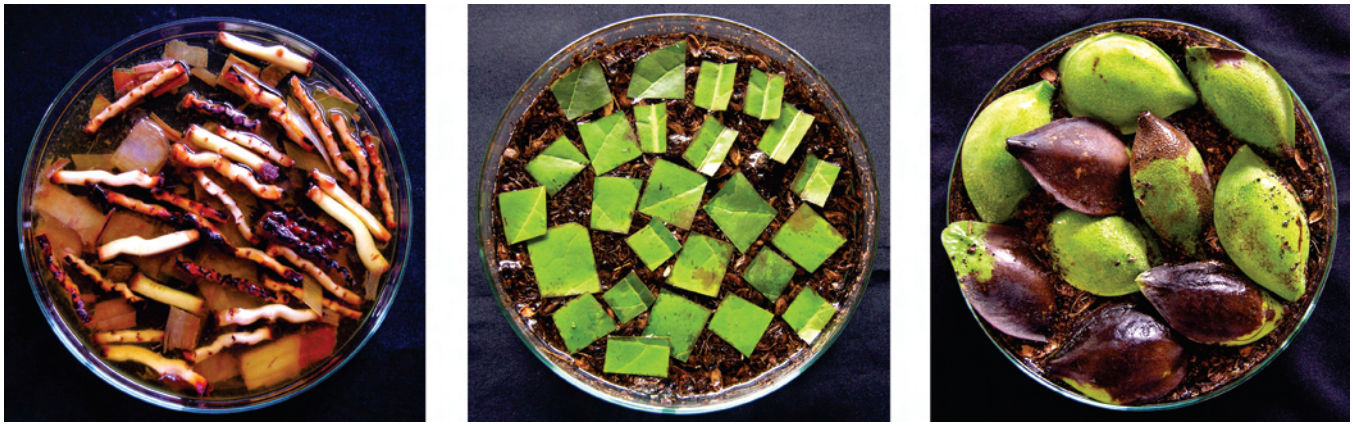

Fig. 2. Isolation of *Phytophthora* species by baiting method

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