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Evaluations of fungicides on *Phytophthora palmivora* incitant of bud rot disease in coconut under *in vitro*

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Abstract
Bud rot disease in coconut is a debilitating disease and is fatal if timely control measures are not taken up. *Phytophthora palmivora* Butl., is the casual agent of the disease. In this study effect of different chemicals *viz.*, Azoxystrobin 23% EC, Kresoxim methyl 44.3% SC, Pyroclostrobin + Metiram 60% WG, Fenamidone + Mancozeb 60% WG, Bordeaux mixture 1%, Copper oxychloride 50% WP, Mancozeb 75% WP, Metalaxyl + Mancozeb, 68% WP and Iprovalicarb + Propineb 6675 WP 5.5% + 61.25% W/W were assessed against *P. palmivora* under *in vitro* conditions. Among the chemicals, Bordeaux mixture 1%, Copper oxychloride 50% WP showed complete inhibition of the mycelia growth of the pathogen after eight days inoculation under *in vitro* screening. Pyroclostrobin + Metiram 60% WG showed 76.67 percent inhibition of mycelial growth followed by Kresoxim methyl 44.3% SC (66.33%) compared with control.

Keywords: *Phytophthora*, chemicals and inhibition

Introduction
Coconut is (*Cocos nucifera* Linn.) the most important perennial fruit plant in the world belonging to the family Arecaceae (Palmaceae). Coconut palms are successfully grown in the tropics and hence referred to as 'King of the tropical palms'. However, many factors such as diseases and disorders can reduce crop yield. Coconut diseases may be classified as those affecting the bud, the leaf, the stem and the root. Some of the diseases are fatal and others are debilitating in nature, one among the major diseases is bud rot. The first visible symptom is wilting of spindle leaf which can be identified from the pale colour of the spindle on careful observation. Later, the spindle turns brown, dries and bends down. Subsequently the tissues surrounding the terminal bud also rot emitting a foul smell. Ultimately, palm succumbs to death (Quillec *et al*., 1984; Nambiar, 1994; Sharadraj and Chandra Mohanan, 2012 and 2013) [6, 4, 9, 10]. *Phytophthora palmivora* was reported as the only species causing bud rot of coconut in India (Rasmi, 2003) [7]. *P. palmivora* also causes rotting and immature nut fall (Nambiar, 1994) [4]. It affects the palms of all ages, but young palms are found to be most vulnerable. The disease is caused by fungus and the incidence is found to be severe during monsoon when the relative humidity is high. With the onset of dry weather the infection becomes less severe and the fungus remains dormant in the leaf base.
The chemical control is being one of the viable propositions to control the disease and to protect the crop (Kumar *et al*. 2003) [3]. Chemical control offers great potential and plays an important role in reducing the losses caused by the diseases (Gill, 1999) [2]. The information regarding the effect of different fungicides against *P. palmivora*, the causal organism of bud rot of coconut is not enough which demand more investigation on testing of new molecules. The present study was undertaken to evaluate the efficacy of some new fungicide molecules against *P. palmivora*, the causal agent of bud rot disease of coconut.

Materials and Methods
Collection of sample and in situ testing
Diseased plant parts of Coconut (*Cocos nucifera*) showing typical bud rot symptoms were collected. The pure pathogen culture (*P. palmivora*) was made by the hyphal tip isolation method (Sinclair and Dhingra 1985) [11] on the PDA medium in Petri plates. The hyphal tips were transferred onto PDA plate after growing the mycelium.

The new plates were incubated at 27 ± 1°C for acervuli production in an incubator. This culture was maintained throughout the study by periodical transfers on (PDA) medium under aseptic conditions, to keep the culture fresh and viable. Advanced hyphae were collected and transferred into the test tube slants containing PDA and incubated at room temperature for seven days. After incubation, the slants were carefully checked for contamination and then preserved at 4°C in a refrigerator for further use.

The efficacy of nine fungicides including systemic and combination products as shown in table 1 were tested against the pathogen under aseptic conditions by employing poisoned food technique (Nene and Thapliyal, 1993) [5]. The experiment was carried out twice in a completely randomized design (CRD) with three replications for each of the three treatments. The per cent inhibition of mycelial growth in each treatment was calculated using the formula suggested by Vincent (1947) [12].

Table 1: The list of fungicides and their test concentration along with formulation used in the present study are given below

S. No.	Treatments	Formulation	Concentration (%)
1	Azoxystrobin	23% SC	0.1
2	Kresoxim methyl	44.3% SC	0.1
3	Pyroclostrobin + Metiram	60% WG	0.2
4	Fenamidone + Mancozeb	60% WG	0.3
5	Bordeaux mixture	1%	1
6	copper oxychloride	50% WP	0.3
7	Mancozeb	75% WP	0.2
8	Metalaxyl + Mancozeb	68% WP	0.4
9	Iprovalicarb + Propineb 6675 WP	5.5% + 61.25% W/W	0.3

Poison food technique

Required quantity of individual fungicide was prepared and added separately in to sterilize molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelial discs of five mm size from seven days old culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. The plate without any fungicide served as control. Three replications were maintained for each concentration. Such plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the formula given by Vincent (1947) [12].

$$I = \frac{C-T}{C} \times 100$$

Where, I = per cent inhibition, C = growth in control, T = growth in treatment

Results and Discussion

In the present investigation nine fungicides were evaluated for their bio efficacy against *P. palmivora* using poisoned food technique at their recommended concentration. Among the fungicides tested, Bordeaux mixture (1%) and copper oxychloride (50% WP) completely inhibited the mycelial growth of the pathogen after eight days of inoculation under *in vitro* conditions compared to control as shown in table 2 and plate 1. Among the eight fungicides tested *in vitro* against a strain of *Phytophthora palmivora* pathogenic to cocoa, cuprous oxide, cycloheximide and mancozeb were highly toxic at low concentrations at most stages in the development of the fungus (Rey and wood, 1983) [8].

Pyroclostrobin + Metiram 60% WG at 0.2 % concentration recorded the mycelial growth of 23.33mm at 8 days after inoculation with 76.67 % inhibition followed by Kresoxim methyl 44.3% SC (66.33%) compared with control as shown in figure 1. Among the 11 fungicides evaluated, Metalaxyl 8% + Mancozeb 64%, Metalaxyl M 4.0 % + Mancozeb 64%, Fenamidone 10% + Mancozeb 50%, Dimethomorph 50% and Cymoxanil 8% + Mancozeb 64% each at 250 ppm, Carbendazim 12% + Mancozeb 63% at 500 ppm and Fosetyl-Al 80% at 3000 ppm completely inhibited the growth of *P. palmivora* under *in vitro* condition (Sharadraj, K.M. and Chandramohanan, R, 2013) [10].

On the basis of present study the effective fungicides Bordeaux mixture and copper oxychloride were proved most effective against *P. palmivora* under *in vitro* conditions followed by Pyroclostrobin + Metiram 60%. This may be due to antifungal activity agents and imparts its poisoning effect on the metabolic process of pathogen. Therefore the growth of *Phytophthora* might be affected.

Table 2: *In vitro* evaluation of latest fungicides against bud rot disease

S. No.	Treatments	Formulation (%)	Concentration (%)	Mycelial growth (mm) of *P. palmivora*
				8 DAI
1	Azoxystrobin	23% SC	0.1	41.67 (40.18)
2	Kresoxim methyl	44.3% SC	0.1	36.67 (37.22)
3	Pyroclostrobin + Metiram	60% WG	0.2	23.33 (28.78)
4	Fenamidone + Mancozeb	60% WG	0.3	46.67 (43.09)
5	Bordeaux mixture	1%	1	0.00 (0.00)
6	copper oxychloride	50% WP	0.3	0.00 (0.00)
7	Mancozeb	75% WP	0.2	60.00 (50.77)
8	Metalaxyl + Mancozeb	68% WP	0.4	51.67 (45.96)
9	Iprovalicarb + Propineb 6675 WP	5.5% + 61.25% W/W	0.3	48.33 (44.04)
10	Control			90.00 (71.57)
	SE(m)±			1.18
	CD (P≤0.05)			3.51

*Values in parenthesis are arcsine transformed

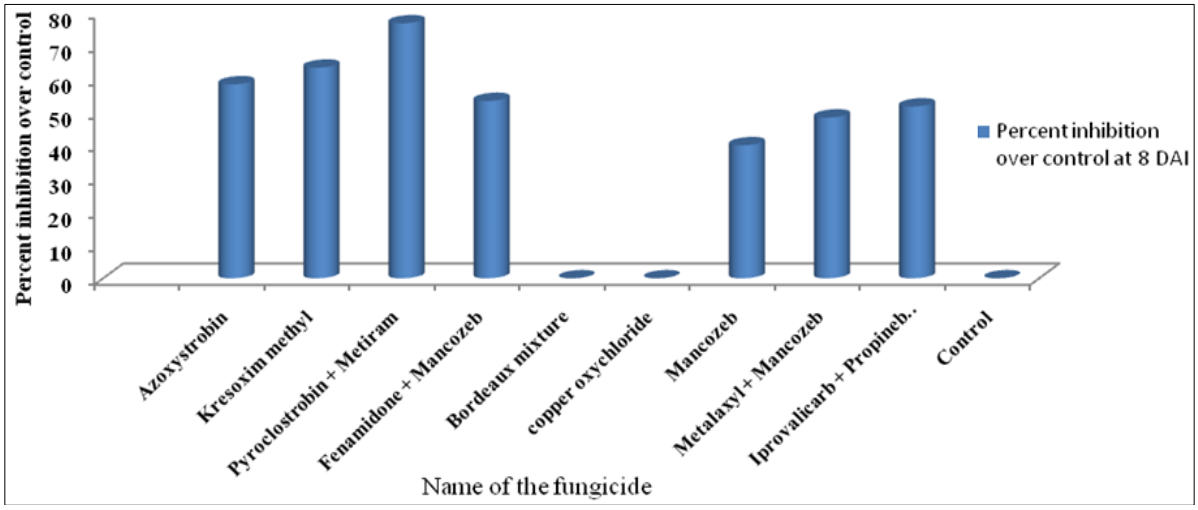

Fig 1: Mean per cent mycelial growth inhibition of *P. palmivora*

Plate 1: *In vitro* evaluation of latest fungicides at recommended concentrations against *P. palmivora*

Conclusion

Use of fungicides for the control of plant diseases is a common practice. As *P. palmivora* is a soil borne pathogen and developed resistant to many of the fungicides. So we have to look for newer fungicides to use these in integrated management practices, Among the tested new molecules, Bordeaux mixture 1%, Copper oxychloride 50% WP showed complete inhibition of the mycelia growth of the pathogen under *in vitro* screening, hence studies were further need to be taken against *P. palmivora* in field to manage the bud rot disease.

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